

to that area; but during the past, this disease has repeatedly crawled out of its filthy lair in the Ganges delta to slither along the trade routes of the world, spreading death and terror among the peoples of all nations, including the United States. Thus, while it may be difficult or impossible to classify diseases strictly into tropical and temperate categories, it is obvious that the tropical regions of the earth do constitute a great reservoir which serves as a breeding place for many of the dangerous diseases of man. These diseases are not only a constant menace to the local inhabitants and to visitors from the temperate zones, but they are a potential hazard to the whole world.

This situation has probably existed during the entire period of man's stay on earth. Nothing is known about the diseases of pre-historic man, but the geologists report that certain insects now recognized as disease vectors were present long before the appearance of the genus *Homo*. They tell us that since man's creation the earth has been passing through one of its periodical ice ages but that during this period warm climatic conditions have always existed in the equatorial regions. The dangers of life in the tropics have been recognized at least since the beginning of historic time. The ancient Greeks divided the earth into five zones and thought that only the two temperate zones were suitable for human existence, the others being either too cold or too hot. This concept persisted and discouraged European exploration of the tropics until late in the Fourteenth Century. During the period of great exploration which followed, vast tropical areas were discovered, conquered, and exploited by Europeans; but, as a rule, these hot regions were not colonized as successfully as were the more temperate parts of the New World. Even today, some of the most sparsely settled areas of the globe lie in the cold polar regions and in the disease-ridden tropics. These regions are not uninhabitable. If freed of their diseases, many potentially rich tropical areas would afford a desirable place in the sun for many of the earth's inhabitants. Thus, the solution of this problem is a continuing challenge to our profession of tropical medicine.

The significance of this statement is obvious when one considers the contribution of tropical medicine to the improvement of world health during the brief period of its development as a special field of medicine. The great medical revolution which started in Europe during the last century led to a rapid accumulation of basic knowledge about the micro-biological sciences—protozoology, bacteriology, virology, and medical entomology. These sciences afforded a sound basis for the phenomenal development of medicine and public health which followed in certain countries of the temperate zone.

The United States affords a good example of this progress. Health conditions here are still far from ideal, but during the present century there has been a great reduction in our disease and mortality rates; and the span of life has been enormously increased. Many enteric diseases are better controlled; and since the New Orleans yellow fever epidemic of 1905, this country has not experienced a serious invasion by any tropical or exotic disease. Therefore, if we except a few infections, including malaria, dengue, and the dysenteries, which still occur in certain localities, the Twentieth-Century experience of Americans with tropi-

THE
AMERICAN JOURNAL
OF
TROPICAL MEDICINE

VOLUME XXVII

EDITED BY
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BALTIMORE, MD.
1947

RESULTS OF CERTAIN STUDIES ON THE STOMACH IN SCHISTOSOMIASIS JAPONICA¹

EDDY D. PALMER²

Until the opportunity to study American soldiers with *Schistosoma japonicum* infections presented itself during World War II, there was little doubt or inquiry into the generally accepted belief that the mature *Schistosoma japonicum* largely inhabit the radicles of the superior mesenteric vein, draining the small intestine. This concept was based on the careful and critical animal experimentations of Faust and Meleney (1), and the assumption that the worms choose similar sites in the human host was propagated because of the lack of accessible reports to the contrary on human autopsy material.

Although postmortem studies on soldiers infected in the southwest Pacific have not yet been published, some investigations have been reported which shed light on the probable distribution of adult *S. japonicum* in the human lower splanchnic area, and which have necessitated some revision of previous ideas on the subject. Johnson and Berry (5) and Hollands and Palmer (4) (in press) found by rectal biopsy and rectal crypt aspiration respectively that *S. japonicum* eggs are shed into the rectal tissues even in early and presumably light infections, thus indicating that the adult worms are ovipositing in radicles of the lower branches of the inferior mesenteric vein. These results and their implications parallel those which Ottolina and Atencio (7), Rincon Urdaneta (8), and Geib and Cheney (3) have obtained by rectal biopsy in the case of *S. mansoni* infections, and show that both species regularly inhabit veins draining the rectum.

On the basis of their work with the Army Commission on Schistosomiasis, Faust, Wright, McMullen and Hunter (2) make this cautious statement: "Previously . . . on the basis of schistosomiasis japonica infections in experimental animals, it has been concluded that in the average infection during the acute stage the mature worms and the lesions for which they and their eggs are responsible are located primarily in the portion of the bowel drained by the upper branches of the superior mesenteric vein The relatively common observance of "yellowish nodules" or distended capillaries in the lower sigmoid colon and upper rectal levels in the American military cases indicates that the conclusion based on findings in experimental animals was somewhat misleading."

Thus, since it has been shown that the lower levels of the gut are involved in the disease, an attempt was made to discover whether those as high in the splanchnic area as the stomach are also affected. To clarify the living pathology of that organ in this infection, gastroscopic and X-ray studies were made on patients with proven active infections at random intervals before and during treatment.

¹ Grateful appreciation is extended to Major Robert A. Hollands, M. C., AUS, who corroborated several of the gastroscopic examinations.

² First Lieut. M. C., A.U.S., Ashford General Hospital, West Virginia.

Three of the non-immunized controls died of acute malaria with high parasitemia eight to nine days after infection and two survived. One of these had a parasitemia never exceeding 0.2% which persisted for 15 days, the other had a low-grade parasitemia which reached a peak of 3.2% on the eleventh day and disappeared on the fifteenth day (fig. 7).

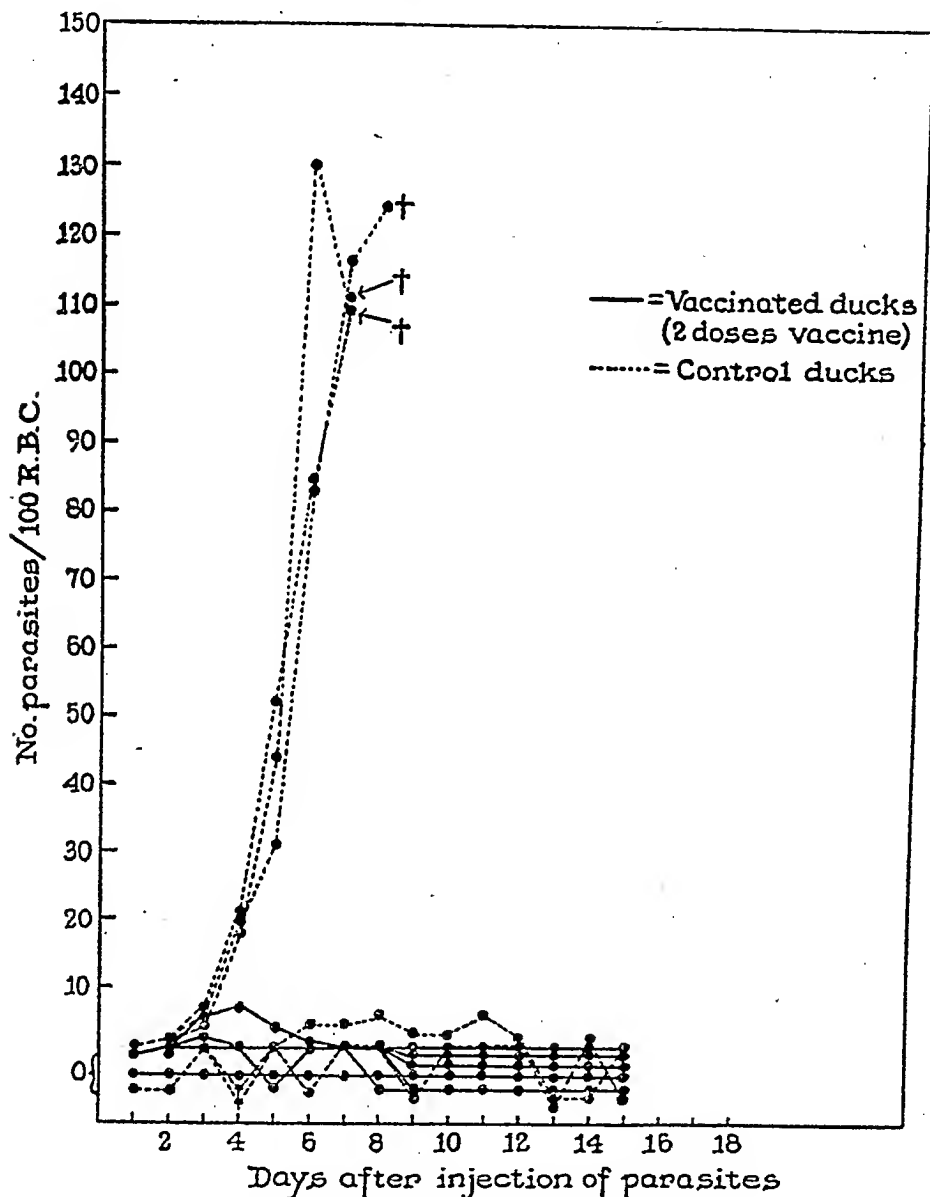


FIG. 7. EXP. 6—CHALLENGE 1 PARASITEMIA.

Of the group of vaccinated ducks receiving two injections of vaccine, none died of malaria (fig. 7). One developed a parasitemia with peak of 6.6% on the fourth day; three developed low-grade parasitemia with peaks of 2.0%, 1.4% and 0.4% and in the other, parasites were never found in blood smear. In this group, the four ducks which showed parasitemia all cleared their blood of parasites by the ninth day and did not show subsequent parasitemia.

CONTENTS

NOTE: The publication of papers the title of which is preceded by an asterisk (*) was made possible by a grant from the Coolidge Foundation to the American Society of Tropical Medicine.

JANUARY, 1947, No. 1

Tropical Medicine and the Challenge of Global War. Presidential Address, 1946, American Society of Tropical Medicine. James Stevens Simmons.....	1
Tropical Medicine and the Challenge of Global Peace. Presidential Address, 1946, American Society of Tropical Medicine. James Stevens Simmons.....	11
*The Distribution of Exoerythrocytic Parasites and the Tissue Reaction Caused by Blood-Induced <i>Plasmodium gallinaceum</i> Infection in Chicks. John L. Tullis.....	21
Studies on Atabrine (Quinaquine) Suppression of Malaria. III. The Epidemiological Significance of Atabrine Suppression. F. B. Bang and N. G. Hairston.....	31
The Transmission of <i>Plasmodium malariae</i> by <i>Anopheles maculipennis</i> Freeborni. Martin D. Young and Robert W. Burgess.....	39
The diagnosis of <i>Schistosoma mansoni</i> Infections. Note on the Use of a Rectal Scraper. Thomas H. Weller.....	41
Results of Certain Studies on the Stomach in <i>Schistosomiasis japonica</i> . Eddy D. Palmer.....	45
Intradermal and Complement Fixation Reactions Elicited by Various Antigens in Persons Infected with <i>Onchocerca volvulus</i> . John Bozicevich, Anthony Donovan, Luis Mazzotti, Francisco Diaz A., and Enrique Padilla.....	51
Intestinal Parasitic Infections in Naval Personnel. Edward K. Markell, Paul E. Mullinger and Donald J. Schneider.....	63
DDT Dust for the Control of Head Lice. Frank A. Cowan, Ted McGregor and Neal M. Randolph.....	67
The Treatment of Carrion's Disease with Large Transfusions. Corrin H. Hodgson...	69
The Evaluation of the Surgical Treatment of Recurrent Echinococcal Cysts of the Liver Followed by Deep X-Ray Therapy. G. M. Dorrance and J. W. Bransfield.....	77

MARCH, 1947, No. 2

Immunization of Ducks against Malaria by Killed Parasites with or without Adjuvants. K. Jefferson Thomson, Jules Freund, Harriet E. Sommer, and Annabel W. Walter...	79
Premature Rupture in Schizogony; an Explanation for Multiple Infection of Red Blood Corpuscles with Malaria Parasites. S. G. Masillamini.....	107
Pacific Vivax Malaria in the American Negro. Fred A. Butler and James J. Sapero.....	111
Fuadin Therapy in 150 Cases of <i>Schistosomiasis mansoni</i> with a Follow Up of 70 Cases. Rafael Rodriguez-Molina and Harry Shwachman.....	117
Parasitologic Study of 400 Soldiers Interned by the Japanese. Ella Louise May, M. T.	129
Agglutination of <i>Endamoeba histolytica</i> Cysts.....	131
Preliminary Report on Field Experiments to Demonstrate Effectiveness of Various Methods of Malaria Control. John E. Elmendorf, Jr.....	135
Radical Cure of Avian Malaria (<i>Plasmodium cathemerium</i>) with SN 8557, a Naphthoquinone Derivative. Wendell Gingrich, Eugenia Worley Schoch, Marjorie Schwab and Catherine C. Shepherd.....	147
*Experimental Chemotherapy of Amebiasis. Hamilton H. Anderson, Herbert G. Johnstone, and Eder L. Hansen.....	153
The reaction of Woolly Opossums (<i>Caluromys laniger</i>) to Yellow Fever Virus. Charles R. Anderson and Manuel Roca-Garcia.....	161

cases outnumbered falciparum cases in both primary and readmission categories. During the last two months approximately 75 per cent of all cases were due to *P. vivax*. The sample month presented in Tables I and II is the eleventh month of figure 1.

Taken as a whole, figure 1 shows that no appreciable difference existed between total negro and white malaria incidence. Primary attack rates were very nearly the same, and while readmission rates were not as parallel they approached quantitative equality.

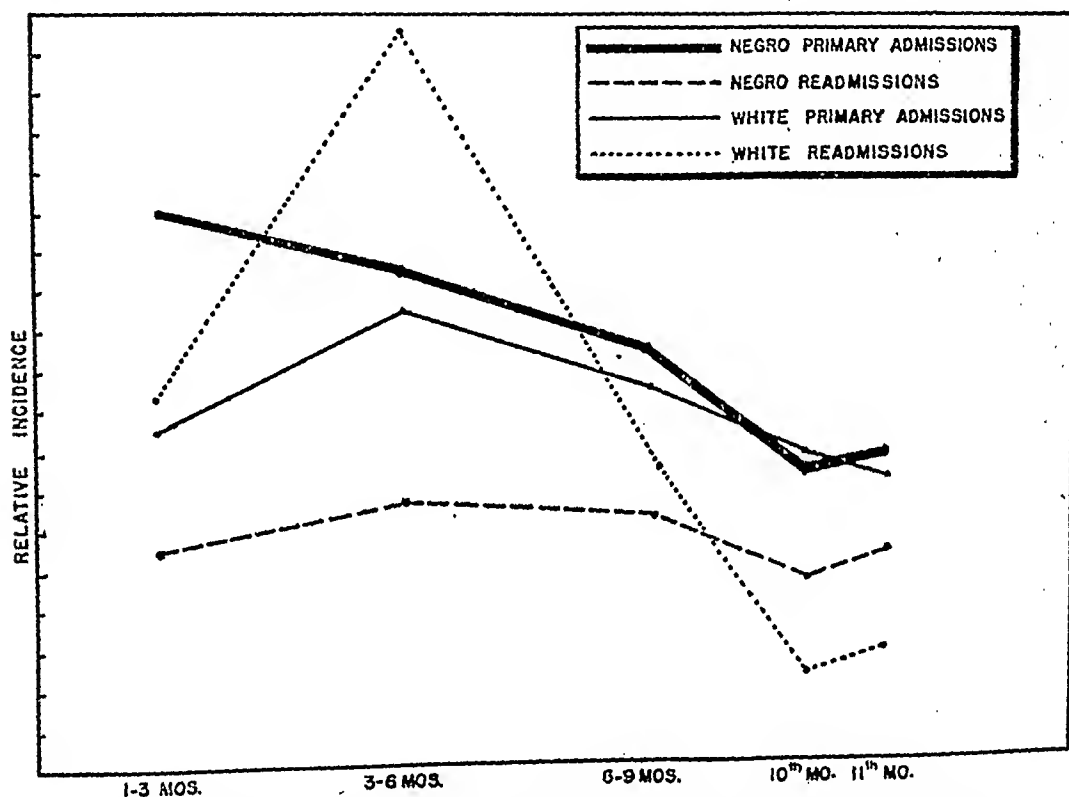


FIG. 1. RELATIVE INCIDENCE OF PRIMARY AND READMISSION MALARIA AMONG WHITE AND NEGRO TROOPS

All clinical and epidemiological observations on the group agree with the foregoing data. Incubation period, clinical severity, response to antimalarial drugs, frequency of recurrences and man days lost were, as nearly as could be determined under routine military conditions, virtually identical among the two races.

It is interesting in this connection to note that native Pacific Islanders (Melanesians) are apparently quite resistant to their local strain or strains of *P. vivax*. According to the reports of colonial physicians, malaria is a frequent and often severe illness among children, but is relatively mild and usually almost symptomless among adults despite an endemic index ranging from 10 to 52 (3) depending upon area. It was found by the authors that atabrine in inadequate suppressive dosage for American troops (0.2-0.4 gms. weekly) was highly effective in reducing the incidence of parasitemia among natives.

Reduction of Anopheles Density Effected by the Pre-season Spraying of Building Interiors with DDT in Kerosene, at Castel Volturno, Italy, in 1944-1945 and in the Tiber Delta in 1945. Fred L. Soper, F. W. Knipe, G. Casini, Louis A. Riehl and A. Rubino.....	177
Book Review.....	201
*The Periodicity of Microfilariae in Two Patients with Filariasis Acquired in the South Pacific. Don E. Eyles, George W. Hunter, III, and Virginia G. Warren...	203
*Infectivity of Pacific Island <i>Wuchereria bancrofti</i> to Mosquitoes of the United States. Don E. Eyles and Harry Most.....	211
Use of Dimethylphthalate Impregnated Clothing as Protection against Scrub Typhus. Louis G. Welt.....	221
*Shigella Carriers with Special Reference to Their Therapy, Including the Use of Streptomycin. D. W. van Gelder, W. P. Daines, and G. L. Fischer.....	225
*The Cuticular Morphology of some Common Microfilariae. Lee Foshay.....	233

MAY, 1947, No. 3

*Tissue Pathology of Experimental Tsutsugamushi Disease (Scrub Typhus) in Swiss Mice and Macacus Rhesus Monkeys and the Report of one Human Case Acquired in the Laboratory. John L. Tullis, Isodore Gersh, Elizabeth Jenney, W. F. McLimans, and J. W. Vinson.....	245
New Concepts in the Treatment of Relapsing Malaria. The Charles Franklin Craig Lecture, 1946. James J. Sapero.....	271
Polyvalent Antigens for the Diagnosis of Salmonellosis in Different Climates. Oscar Felsenfeld.....	285
The Geographical Distribution of Shigella. Viola Mae Young.....	293
Attempts to Cultivate the Mosquito Phase of <i>Plasmodium relictum</i> . Gordon H. Ball.	301
Clinical Standardization of Pamaquin (Plasmochin) in Mosquito-Induced Vivax Malaria, Chesson Strain. Branch Craig, Jr., Ralph Jones, Jr., Merrill Whorton, Theodore N. Pullman, Alf S. Alving and Lillian Eichelberger.....	309
The determination of the Folie Acid Content of Foods Usually Consumed by Patients with Tropical Sprue. Juan J. Angula, and Tom D. Spies.....	317
Isospora Hominis Infection in Man. Ella Louise May, M. T.....	323
Treatment of trichuriasis with 'Enseals' of Emetine Hydrochloride. Robert B. Burrows, William G. Morehouse and Joe E. Freed.....	327
Piperonyl Butoxide, A New and Safe Insecticide for the Household and Field. Walter E. Dove.....	339
Schistosomiasis japonica. Samuel S. Leavitt, Capt. M. C., AUS., and Otis H. Beek, Capt. M. C., AUS.....	347
Arthropods of Sanitary Importance in the Republic of Nicaragua, Central America. Paul A. Woke.....	357
*Actinomycosis in a <i>Hydrochoerus isthmius</i> Goldman (Isthmian Capybara or Poncho). Carlos Calero M., and Pedro Ortiz O.....	377
Tropical Phagedenic Ulcer (Vincent's Ulcer). Harvey Blank.....	383
Experimental Studies with <i>Pasteurella tularensis</i> in Fish. Banner Bill Morgan.....	399
The Fate of Radioactive Tartar Emetic Administered to Human Subjects. I. Blood Concentration and Excretion Following Single and Multiple Intravenous Injections. F. C. Bartter, D. B. Cowie, Harry Most, A. T. Ness, and Scott Forbush...	403

SUPPLEMENT TO No. 3, MAY, 1947

*Malaria and Other Insect-Borne Diseases in the South Pacific Campaign, 1942-1945.	
I. General Aspects and Control Measures. P. A. Harper, E. T. Lisansky and B. E. Sasse.....	1
II. Epidemiology of Insect-Borne Diseases in Army Troops. W. G. Downs, P. A. Harper and E. T. Lisansky.....	69

necrotic tissue in which it was not possible to establish any cellular differentiation. There was congestion of the lung vessels around the tumor.

We did not see any pathology in the lymph nodes and spleen with the exception of marked eosinophil infiltration.

The examination of a piece of striated muscle fibres showed a large Sarcocystis of about 0.4 ctns., with the typical microscopic arrangement reported (5), as was identified by Dr. Herbert C. Clark of this laboratory.

Animal Inoculation: With the findings previously reported several portions of the tumor-mass located under the skin of the right fore-foot and liver (which had been preserved for 10 days in a 10% formalin solution) were washed in running water to be cultured and inoculated, in spite of the fact that negative results have been reported in similar conditions. At the end of this period, the

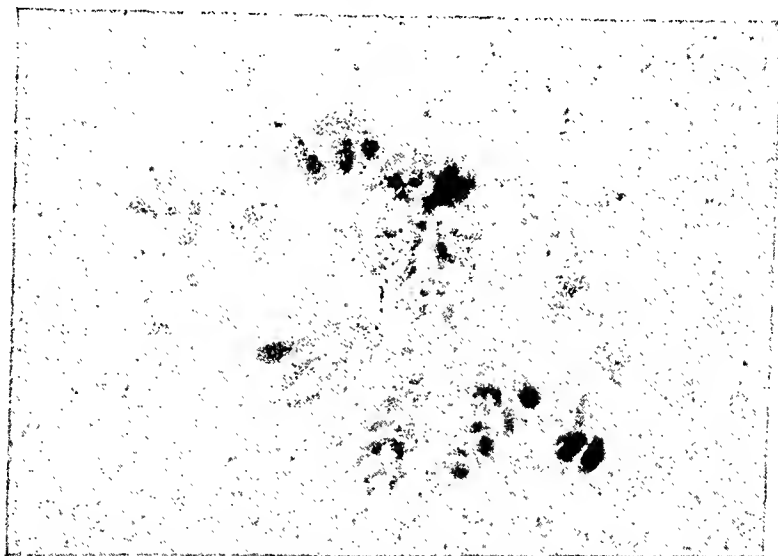


FIG. 3. GRAM NEGATIVE ACTINOBACILLIFORM BODIES. $\times 750$

tissues were triturated in normal saline solution and 1 c.c. of fine suspension was inoculated subcutaneously in a rabbit, a mouse and a guinea pig; and equal doses were injected intraperitoneally in a mouse and a guinea pig.

The investigation, as might have been expected, was negative.

Culture: A portion of the triturated tumor tissue located under the skin of the right fore-foot was inoculated in Sabouraud's prouve medium and incubated at room temperature (between 26 and 30 degrees C.), aerobically and anaerobically. Daily observations were made of the plates for 28 days. The investigation was negative.

Staining characteristics: Examination of the organism encountered regarding its staining properties with the colorants commonly used in pathology (hematoxylin and eosin) showed (fig. 1), that the central area of what resembled Ray-fungus, consisting of the round sporocytic bodies, stained deeply with hematoxylin; and that the peripheral portion, formed of the actinobacilliform rods, had a marked affinity for eosin.

It was also demonstrated that the organism was (fig. 3) Gram-negative (6) and acid-fast.

III. Entomology. P. W. Oman and L. D. Christenson.....	91
IV. Parasitological Observations on Malaria in Natives and Troops, and on Filariasis in Natives. Norman D. Levine, and Paul Harper.....	119

JULY, 1947, No. 4

*The Epidemiology of Schistosomiasis Japonica in the Philippine Islands and Japan. III. Surveys of Endemic Areas of Schistosomiasis Japonica in Japan. Williard H. Wright, Donald B. McMullen, Harry J. Bennett, Preston M. Bauman, and James W. Ingalls, Jr.	417
Block Residual Spraying of Premises with DDT for the Control of Malaria. E. Harold Hinman and Lawrence K. Cutkomp.....	449
Observations on the Laboratory Care, Life Cycle, and Hosts of the Chicken Mite, <i>Dermanyssus gallinae</i> . Charles L. Wisseman, Jr., and S. Edward Sulkin.....	463
Studies on the Cyclic Passage of Yellow Fever Virus in South American Mammals and Mosquitoes. III. Further Observations on <i>Haemagogus equinus</i> as a Vector of the Virus. Mary B. Waddell, and R. M. Taylor.....	471
Psychotic Reactions to the Ingestion of Large Doses of Quinacrine in Normal Subjects. Sibley W. Hoobler.....	477
The Lymph Node in Tropical Diseases. Colonel J. E. Ash, MC., U.S.A.....	483
The Effectiveness of Metachloridine in Suppressing Natural Infections with <i>Plasmodium malariac</i> and <i>P. falciparum</i> in British Guiana. Michael Kenney and Sterling Brackett.....	493
Further Note on the Classification of Vibrios of the 1945 Cholera Epidemic in Chungking. Hobart A. Reimann.....	503
The Present Status of Hookworm Infection in Florida. Marion Hood.....	505
Liberia, Scene of New International Institute for Tropical Medicine.....	517
The Fourth International Congresses on Tropical Medicine and Malaria.....	521
Books Received.....	525

SEPTEMBER, 1947, No. 5

Oral Emetine in the Treatment of Intestinal Amebiasis. Bliss C. Shrapnel.....	527
Alcoholic Extract Medium for the Diagnosis and Cultivation of <i>Endamoeba histolytica</i> . E. Clifford Nelson.....	545
The Metabolism of Chiniofon using Radioactive Iodine. Edwin C. Albright, D. L. Tabern, and Edgar S. Gordon.....	553
*Types of American Cutaneous Leishmaniasis—Dermatological Aspects. A Review. Leon Goldman.....	561
*Some Characteristics of Foreign Vivax Malaria Induced in Neurosyphilitic Patients. Martin D. Young, John M. Ellis and Trawick H. Stubbs.....	585
The Molluscan Intermediate Host and Schistosomiasis japonica. III. Experimental Infection of <i>Oncomelania quadrasi</i> , The Molluscan Intermediate Host of <i>Schistosoma japonicum</i> . Maj. George W. Hunter, III, PC. Maj. Harry J. Bennett, Sn.C., T/Sgt. James W. Ingalls, Jr., and Cpl. Eugene Greene.....	597
The Rectoscopic Biopsy by Transparency. A New Diagnostic Method for <i>Schistosoma mansoni</i> . Carlos Ottolina.....	603
Filarial Infection in Costa Rica. Donald C. A. Butts.....	607
*Reactions of Natives of Okinawa and of American Personnel to Skin Tests with Test Antigen Prepared from Microfilariae of <i>Dirofilaria immitis</i> . M. B. Franks, B. M. Chenoweth, Jr., and N. R. Stoll.....	617
Evaluation of Penicillin in the Treatment of Yaws. Final Report. Albert J. Sheldon, James D. Dwinelle, Charles T. Rein, and Thomas H. Sternberg.....	633
*Salmonella Infections in Panama. Review of 219 Consecutive Hospital Cases Occurring in the 5 Year Period 1942-1946. Lowell L. Henderson.....	643

population in the places named; the stool examinations were conducted with the antiformin-ether technique, which is much more efficient than is the technique using a single fecal smear.

In addition to the surveys listed in table 1, a total of 2,678 persons was examined in Toyosato village and 1,536 persons examined in Takaoka village, Katori County, in November and December, 1940, without any being found positive for schistosomiasis.

In Higashikatsushika County, 2,751 persons were examined in the village of Okashiwa in January, 1941, without any positives. Apparently no surveys have been made since that time.

TABLE 2

Incidence of S. japonicum and other helminth parasites in school children (ages 8 to 14 years) examined by Commission on Schistosomiasis in Tone River area, Chiba and Ibaraki Prefectures

SCHOOL	NO. EXAM- INED	NO. MALES	NO. FE- MALES	NO. NEGA- TIVE	NUMBER INFECTED								
					<i>S. japonicum</i>		<i>A. lum- bri- coides</i>	Hook- worm	<i>T. tri- chiura</i>	<i>E. ver- micu- laris</i>	<i>H. nana</i>	<i>Clonor- chis si- nensis</i>	<i>Metag- onimus sp.</i>
					Males	Fe- males							
Sakura (Sakura Town)	68	31	37	33	1	0	24	17	5	1	1		
Sakura (Wada Village)	46	17	29	24	0	0	13	8	3	2	1		
Sakura (Nego Village)	51	29	22	25	0	0	18	7					
Toyoshima	76	25	50	20	1	0	49	7	5	2		3	
Kita-Sawara	81	37	44	8	0	1	72	4	16			5	
Moriyama	68	25	37	12	0	0	47	10	4	2		5	2
Totals	390	164*	219*	122	2	1	223	53	33	7	2	13	2

*Sex unknown — 7.

Examination of school children in the Tone River area. Stool samples were obtained from children in four schools in the Tone River area; two of these schools, Toyoshima and Kita-Sawara, are in Ibaraki Prefecture, while the other two, Sakura and Moriyama, are in Chiba Prefecture. The results of the stool examinations are given in table 2. It will be noted that the stool samples from the Sakura School were divided into three sections, those from children from the town of Sakura and those from children from the villages of Wada and Nego. These villages are a few miles from Sakura in an area in which schistosomiasis was supposed to be endemic. However, no infections were found in the children from these villages, and it is possible that the infection has died out or is at a very low ebb in these localities.

Three cases of *S. japonicum* infection were found in a total of 390 individuals

Carrion's Disease. A Study of the Incubation Period in Thirteen Cases. William E. Ricketts.....	657
Division of Tropical Diseases, National Institute of Health.....	661

NOVEMBER, 1947, No. 6

"Mal del Pinto" or "Carate" and its Treatment with Chlorhydrate of 3-Amino-4 Oxiarsenbenzen (Mapharsen). Gerardo Varela and Carlos Avila.....	663
Some Factors that Influence the Degree of Parasitemia in Ducks Infected with <i>P. lo-phurae</i> . R. H. Rigdon and B. E. McCain.....	673
Plague: A Survey of Recent Developments in the Prevention and Treatment of this Disease. James M. Ruegesegger and Huntington Gilchrist.....	683
Method for Testing Ointments and Fabrics to Determine their Effectiveness as Barriers to Schistosome Cercariae. Robert E. Kuntz, M. A. Stirewalt, and J. R. Buchheit..	691
Visceral Leishmaniasis Complicated by Severe Anemia—Improvement Following Splenectomy. Joseph H. Burchenal, Ralph F. Bowers and Thomas A. Haedicke.....	699
The Significance of <i>Endameba histolytica</i> in Stools of Individuals with Acute Diarrhea of Moderate Severity. H. T. Blumenthal, Frank N. Dutra, Howard Paschal and L. R. Kuhn.....	711
A Case of Sprue Maintained on Folic Acid. Edward B. Vedder.....	723
*Sequelae of Japanese B Encephalitis. Thomas W. Simpson, and Gordon Meiklejohn..	727
<i>Aedes aegypti</i> Control in the Absence of a Piped Potable Water Supply. P. F. de Caires	733
Asymptomatic Toxoplasmosis. B. H. Kean and Robert G. Grocott.....	745
Experimental Sporotrichosis in Mice. Roger D. Baker	749
Benzene Hexachloride for Area Control of Trombiculid Mites. J. P. Linduska and F. A. Morton.....	771
Bylaws of the Fourth International Congresses of Tropical Medicine and Malaria, Washington, D. C. May 10-18, 1948.....	779
Books Received.....	787
Book Reviews.....	789

be Sarcosporidia but which we reclassified tentatively (5) as *Toxoplasma*, were found within the muscle. A severe myositis was present. Since chorioretinitis is a feature of congenital toxoplasmosis it may be noted that the patient's vision was described as defective although the description of the ophthalmologic examination is too vague for adequate interpretation. Recently Syverton and Slavin (9) reported a similar case in which toxoplasmas were demonstrated in the gastrocnemius muscle of a 65 year old male ill of a disease resembling typhoid fever. An inflammatory reaction was present in the muscle. These two cases may belong in the group of adult typhus-like toxoplasmosis as described by Pinkerton and Henderson (10) but cannot be appropriately included in a table of asymptomatic toxoplasmosis.

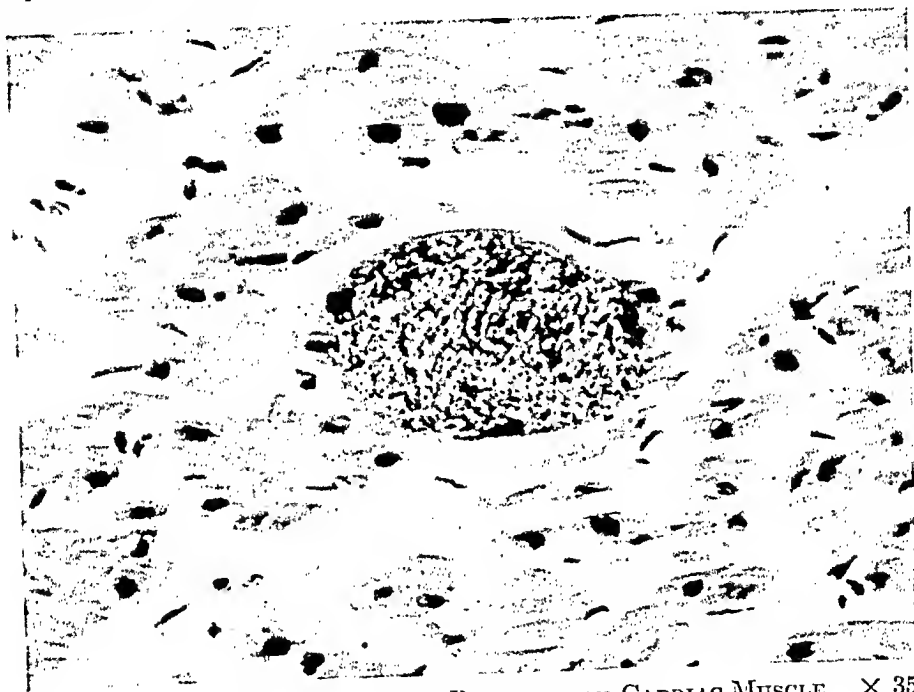


FIG. 1. CYST-LIKE AGGREGATE OF PARASITES IN CARDIAC MUSCLE. $\times 350$

In cases of congenital toxoplasmosis in which the infection in the fetus is widespread, destructive, and often fatal, the mother is apparently free of symptoms. It must be assumed therefore that the parasite can dwell in the human host without causing recognizable lesions. The site of predilection for parasites in fetal tissue is the brain, although almost any organ may be invaded. In these asymptomatic cases, however, the parasites have generally been found in the heart or skeletal muscle alone and attempts to demonstrate parasites in other organs were unsuccessful. Tomlinson (6), however, first found parasites in the brain of a 13 year old child who died of sickle cell anemia and then in a restudy of the heart found *Toxoplasma* pseudo-cysts.

The absence of an inflammatory reaction to the parasite is noteworthy. In most congenital cases an intense inflammatory reaction is present; however, in one premature infant who survived for 25 days after birth inflammatory changes

TROPICAL MEDICINE AND THE CHALLENGE OF GLOBAL WAR¹

JAMES STEVENS SIMMONS²

Fellow Members of the American Society of Tropical Medicine, Distinguished Guests, Ladies and Gentlemen:

INTRODUCTION

I am glad to have this opportunity to thank you for selecting me as your President and to assure you that I appreciate the honor of serving at this Forty-Second Annual Meeting.

This should be an important meeting, for it comes at the most critical period in history—a period when decent people everywhere are rejoicing over the end of World War II and are planning hopefully, but fearfully, for a firm, lasting peace.

It is our second meeting since V-J Day, but the first to be held during the new Atomic Era. As you know, this Era is characterized by a general fear that civilization may be destroyed through the misuse of atomic power and by serious doubts as to whether man has the intelligence to control this new power peacefully and safeguard his own future. These fears are real and well founded; and thoughtful persons of all nations are searching earnestly for a practical solution of the problem. Statesmen are trying to solve it by international agreements, but their efforts are hampered by ancient ingrained hatreds, jealousies and distrust. So far, it appears that the most promising approach to mutual understanding and peace lies in the field of international health. The importance of physical and mental health is universally recognized, and no one doubts the integrity of the unselfish men and women of every nation who are devoting their lives to the humane profession of public health. Therefore, as members of this important profession, we of the Society of Tropical Medicine now have an opportunity to contribute to the security of man's position as an intelligent, peaceful, civilized animal.

During the four decades of its existence, our Society has assisted materially in extending the frontiers of scientific knowledge in the field of tropical medicine and has contributed much to the health and welfare of the human race. Obviously, we must be prepared to meet the problems of the future. Therefore, I feel that this meeting has a special significance for all of us, for it affords a unique opportunity to re-examine our objectives and to re-dedicate ourselves to the difficult task that lies ahead.

In keeping with this spirit, I have selected for today's Presidential address the subject, "Tropical Medicine and the Challenge of Global War", which will be followed at the meeting of the Academy this evening by an address entitled, "Tropical Medicine and the Challenge of Global Peace."

In the present talk, I wish to emphasize the fact that throughout the ages the

¹ Presidential address, delivered at the luncheon of the American Society of Tropical Medicine, Miami, Florida, November 6, 1946.

² Brig. General, U. S. Army; Dean, Harvard School of Public Health.

tropics have constituted a vast reservoir of disease, which has always been a menace to world health, and to show that by meeting the challenge of these diseases in the past tropical medicine has already made a significant contribution to our health and security, especially under the trying conditions presented by the recent World War.

The full importance of the wartime contribution of tropical medicine can be visualized by keeping in mind the wide field covered by this branch of medicine and the magnitude of the military health problems presented by the tropical diseases. The term "tropical medicine" is used here in its broadest sense. It refers to all aspects of medicine and public health as they apply to conditions in the tropics. Obviously, it includes the diagnosis, treatment, and prevention not only of the diseases indigenous to such regions but to a host of others which may exist anywhere but which flourish best and are most prevalent in the hot parts of the world.

We are all familiar with the fact that certain infections, such as African Trypanosomiasis, are limited to specific tropical areas because the conditions necessary for their spread—in this case, the tsetse fly—do not exist elsewhere. Other insect-borne diseases, including yellow fever, malaria, and dengue, are most common in their tropical reservoirs, where climatic conditions favor their propagation throughout the year; but they can and have spread to certain temperate regions, where their vectors exist during warm seasons. Thus, malaria, which is most prevalent among the people of the tropics, is also widespread in many temperate countries. In fact, it still causes more disability and death than any other known infection. Malaria was once common in all parts of the eastern United States, and even today it presents an important health problem in certain southern states. Yellow fever is another dangerous tropical disease which is a continuing menace to world health. At present, it is confined largely to its two vast jungle domains in South America and Africa, and it has not appeared in this country since 1905. During the past, however, this disease was a frequent summer invader of temperate zone ports, and it caused serious epidemics as far north as Philadelphia, New York, and Boston. Dengue fever has long been endemic in many temperate regions, including the southern United States.

The tropical zone is not only a productive incubator of these and various other insect-borne diseases, but it is a spawning place for the innumerable filth diseases which are spread by contact or by the ingestion of contaminated food or drink. Tropical skin infections undoubtedly result in part from climatic conditions. However, in this case, as with most of the filth diseases, a more important factor is the low standard of sanitation and hygiene that prevails generally among the impoverished, backward peoples of many tropical regions. Yaws is a widespread tropical disease. Leprosy was formerly prevalent in Europe, but is now most common in the tropics. The enteric infections are also well-known examples. The dysenteries and typhoids may occur in any part of the world. Their incidence has been markedly reduced in many temperate-zone countries, but they are still the curse of most tropical places. Cholera is still a potential wholesale killer in tropical Asia. At present, it happens to be confined largely

cal diseases, prior to World War II, occurred largely outside the United States and among those who travelled to tropical regions for social, industrial, or military reasons. As a consequence, Americans as a group gradually lost interest in tropical medicine and by 1940, many of our physicians, especially those living in northern states, were convinced that this specialty had lost much of its former importance to American medicine. It was commonly held that in this country tropical medicine had worked itself out of a job and had become a matter of concern only to the less fortunate inhabitants of southern climates. The fallacy of such shortsighted thinking was made apparent by our national experience during World War II.

TROPICAL MEDICINE DURING WORLD WAR II

Tropical medicine played a vital role in the winning of that war. There has never been a time when so many soldiers and sailors from temperate countries were engaged in active military operations under conditions of such widespread exposure to tropical diseases. When the story is finally told of the total contribution of tropical medicine to Allied victory, it will constitute one of the great epics of scientific medicine.

The medical profession of the United States played an active part in this Allied health program, and every medical agency of this country, both civilian and military, contributed to its success. The Army and Navy were intimately concerned since they were faced with the emergency problem of protecting American soldiers and sailors against the disease hazards of practically every region of the world. You are all familiar with the wonderful work of the Navy during the war. I should like to discuss this contribution now, but as I was more intimately concerned with the Army program we will use it as an example of the operations of the armed services. The experience of the U. S. Army alone is sufficient to indicate the broad scope of the contribution made by tropical medicine during the war, for similar programs were carried out by the U. S. Navy and by the armed forces of certain of the other Allied nations.

The active planning for the tropical-disease-control program of the Army began early in 1940. It has as its objectives: first, the development of an effective organization to protect American troops against the diseases of the tropics at home and abroad; and second, the development of adequate safeguards to prevent the introduction of exotic diseases into the United States.

As the scene of our military activities reached out, first, into the Caribbean and, subsequently, into Africa, India, and the islands of the Pacific, the diseases of these regions constituted an increasingly important military problem. The experience gained in meeting this problem should be of great value to the medical profession, for it affords a pattern for future planning. The first need was for a special organization in the Office of the Surgeon General to plan the tropical disease-control program. For this, specialists were obtained in various fields, including internists, parasitologists, bacteriologists, entomologists, sanitary engineers, and others. Civilian specialists were mobilized as consultants and

advisors to assist in developing and operating the program. The American Society of Tropical Medicine furnished many of these workers.

Briefly, the program had the following broad objectives: (1) The collection of information about the disease hazards of all regions in which American troops might be exposed and the use of this information for planning and for the instruction of troops sent abroad, (2) the training of military personnel in the prevention and treatment of tropical diseases, (3) the development of a research program to provide the new basic information and materials required for the military practice of tropical medicine, and (4) the energetic application of all the available information in order to meet the tropical disease problems of the war.

The collection of information about the distribution of disease was organized as a part of the Medical Intelligence Division of the Preventive Medicine Service.

The training of military personnel presented a more serious problem. As a large proportion of the army physicians were recruited from civil life, they were confronted with tropical-disease situations with which they had had little or no experience. As already mentioned, the profession as a whole had lost interest in tropical medicine, and in many of our medical schools the teaching of this subject was either inadequate or altogether lacking. At first, the Surgeon General attempted to meet this deficiency by developing special short courses in tropical medicine for medical officers after they entered the Service. However, only a small fraction of the military physicians in need of such instruction could be reached in this way. Therefore, a longer ranged program was planned. Arrangements were made through the National Research Council to secure a grant to improve the teaching of tropical medicine in the civilian medical schools engaged in training officers for the Army and Navy. Through this grant, 63 of our 77 medical schools sent members of their faculties for short courses in tropical medicine at the Army Medical School, at Tulane, or elsewhere, and many of these men were later given field experience in various parts of tropical Central America. This was done with the hope that upon their return to their respective medical schools they would assist in the teaching of tropical medicine to students preparing for military service. This training program was of real value, and it did much to create a new interest in tropical medicine in America.

The medical research program was one of the most extensive ever launched by a military force, and it produced valuable information which will be of lasting benefit to humanity. The Army program was carried out by both military and civilian scientists. It was closely co-ordinated with the programs of the Navy and the U. S. Public Health Service, and all these were supplemented and spear-headed by the joint medical research program sponsored by the National Research Council and the Committee on Medical Research of the Office of Scientific Research and Development. This national research effort provided many new and effective methods and agents for the treatment and control of tropical diseases. Protective clothing, repellents, and insecticides were made available for the field attack on insect-borne diseases. New drugs, including the sulfonamides

and penicillin were developed for the treatment of many types of infections. Improved vaccines were used against yellow fever, epidemic typhus, tetanus, Japanese B Encephalitis, and influenza; and new therapeutic drugs were found and used for the suppression and treatment of malaria. The application of these and the many other results of this research program contributed directly to the conservation of American manpower and to allied victory.

The value of the Army's wartime tropical medicine program is shown by the official records of the incidence of tropical infections.

American troops were in contact with exotic diseases in all parts of the world, but the record established in preventive medicine was spectacular. Millions of soldiers were exposed to louse-borne typhus in Africa, Asia, and Europe, but they

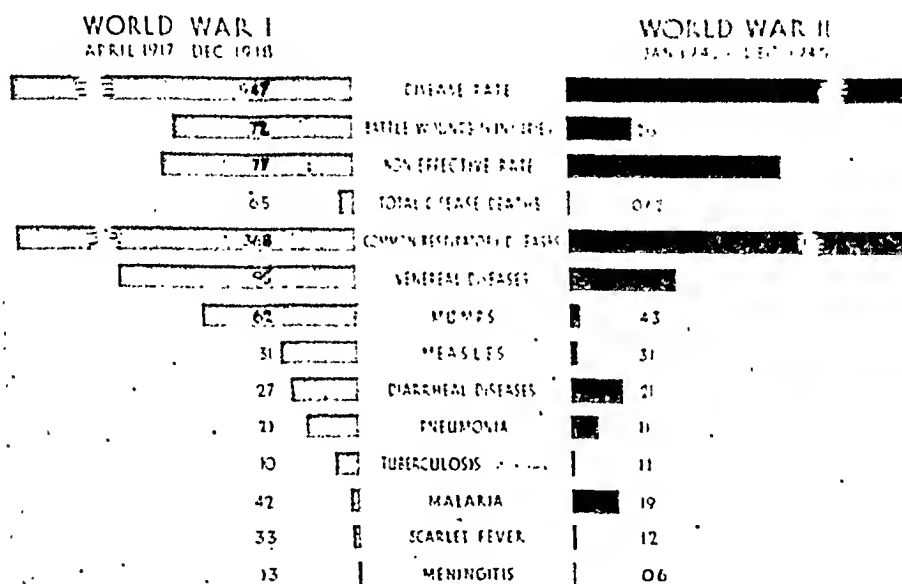


CHART 1. THE DISEASE INCIDENCE RATES OF THE ARMY IN WORLD WAR I AND WORLD WAR II
(Rates per 1000 troops per year)

(Courtesy U. S. Army Medical Museum)

were protected by typhus vaccine and DDT louse powder. Thanks to military sanitation and cholera vaccine, thousands of soldiers served without infection in the cholera-infected cities of India. Vaccines, rodent control, and DDT were used successfully to protect troops exposed to civilian epidemics of bubonic plague in Africa and elsewhere. Military personnel passing through endemic yellow fever areas in South America and Africa were immunized with yellow fever vaccine.

The Army was protected against these and other diseases, including smallpox and the typhoid fevers. Still others, including yaws, leprosy, and African sleeping sickness, failed to appear.

The relative incidence of the tropical diseases that did occur is indicated in Chart 2.

There was not a single case of either yellow fever or plague among our millions of troops. There were only two cases of trypanosomiasis, 13 of cholera, and a few hundred each of relapsing fever and leishmaniasis.

The Schistosomiasis infections, numbering less than 2000, were contracted in the Philippines, largely among personnel of the Engineer and Signal Corps, whose duties required their exposure in infested waters.

Filariasis was responsible for only about 2000 hospital admissions. These infections were contracted early in the war on a few small islands of the South

INCIDENCE OF TROPICAL DISEASES WORLD WAR II HOSPITAL ADMISSIONS

DISEASE	NUMBER CASES	RATE PER 1000 TROOPS PER YEAR
MALARIA	460,872	18.9
DENGUE	84,090	3.4
SANDFLY FEVER	12,434	0.5
SCRUB TYPHUS	6,861	0.28
FILARIASIS	2,151	0.09
SCHISTOSOMIASIS	1,636	0.07
LEISHMANIASIS	307	0.01
RELAPSING FEVER	220	0.01
CHOLERA	13	0.001
TRYPANOSOMIASIS	2	0.0001
YELLOW FEVER	0	0.0000
PLAGUE	0	0.0000

CHART 2. TROPICAL DISEASES IN THE U. S. ARMY

(Courtesy U. S. Army Medical Museum)

Pacific before adequate mosquito control measures were enforced. The cases were mild and there were no serious permanent complications.

Scrub typhus caused less than 7000 admissions, but it was of considerable military importance in parts of the Western Pacific and in Burma. In different outbreaks, the mortality varied from 1 to 30 per cent. Effective methods were developed by the United States of America Typhus Commission for the protection of troops against this disease, by the burning of campsites to destroy the mite vectors and the use of dimethyl phthalate and other miticidal agents for individual protection.

Sandfly fever was responsible for about 12,000 admissions, and dengue fever for about 84,000. Both are mild, non-fatal, self-limited diseases. Their mili-

tary importance is due to the fact that in susceptible populations they tend to occur in explosive, epidemic form. The Army Epidemiological Board developed a vaccine for dengue, and DDT was used to control the phlebotomus vectors of sandfly fever and the mosquito vectors of dengue.

Malaria was the most important tropical disease faced by American troops. There were 460,800 hospital admissions, a rate per annum of 18.9 per 1000 troops. These admissions included relapses and do not indicate the actual number of persons infected. About $\frac{5}{8}$ of the admissions were to hospitals overseas; and the hospital admissions in this country consisted largely of patients with relapses from overseas infections. Deaths were rare.

The malaria control program in the continental United States was highly effective. The Army's program of mosquito control inside military reservations, which cost about 17 million dollars, was supplemented by the program of the United States Public Health Service in war areas at a cost of more than 19 million. As a consequence, the Army's annual admission rates for malaria contracted in this country were negligible, and they decreased progressively each year of the war. Only about 4000 cases were contracted in the United States during the entire war.

The overseas experience was worst early in the war when, because of the military situation, troops fought in highly malarious regions without adequate malaria-control supplies or protection against mosquitoes. Later, when control organizations were firmly established, when supplies became adequate and soldiers were better disciplined in personal protective measures, the malaria rates decreased. In 1945, for example, the total overseas rate for malaria was 25 per 1000 men compared with the peak of 160 in 1943. The new agents developed for malaria control, including such drugs as "Chloroquine" and new insecticides, particularly DDT, not only render military field control more effective, but they afford important weapons for civilian use.

Other tropical diseases encountered by troops include the diarrheas and dysenteries, which caused considerable loss of time but relatively few deaths; and the skin diseases, which were annoying but usually not dangerous.

The Army program was not only concerned with the protection of troops but with the protection of the United States against invasion by exotic diseases. Careful plans were made to afford such protection and barriers against disease were set up all along the line from the tropical theaters to the separation centers here at home. At each barrier, the soldier was examined; and, if necessary, he was treated to avoid spreading disease to civilian communities. An important part of this program was the work of the Inter-Departmental Quarantine Commission established in co-operation with the Navy and the United States Public Health Service. On the whole, these barriers against invasion were effective, and the few diseases that have slipped through have been promptly identified and controlled. Consequently, there have been no serious epidemics traceable to American soldiers or sailors returned from overseas.

This wartime experience with tropical diseases was a jolt to the complacency of those who formerly assumed that tropical medicine was no longer of importance

to the United States. It showed that the diseases of the tropics are still a hazard to Americans who travel or live in certain foreign countries. It showed that this country is still exposed to invasion by exotic diseases and indicated the importance of modifying quarantine procedures to meet the new methods of transportation. Finally, it emphasized the urgent need for a continuing national program of research and training in order to control tropical diseases, both here in the United States and in their tropical reservoirs.

In closing, it should be emphasized that we are still concerned with tropical medicine. The American Medical profession has met the challenge of the diseases of the tropics under the trying conditions of war. It can and must continue to meet this challenge during the future years of peace.

TROPICAL MEDICINE AND THE CHALLENGE OF GLOBAL PEACE¹

JAMES STEVENS SIMMONS²

Members of the American Academy of Tropical Medicine, Distinguished Guests, Ladies and Gentlemen:

INTRODUCTION

I appreciate deeply the honor of serving as your President this year and am delighted to be with you at this Thirteenth Annual Meeting.

It is always a pleasant, stimulating experience for me to attend these joint meetings of the American Academy and the American Society of Tropical Medicine. These gatherings afford an opportunity to meet old friends and make new ones among the members of both organizations who come back each year from the seven seas and the far corners of the earth to discuss the results of their scientific labors. They also provide an opportunity for us to take stock of the progress made in tropical medicine, to re-define our objectives, and to plan for the continuation of effective leadership in this important branch of medicine and public health.

In my Presidential address before the American Society of Tropical Medicine this morning, I indicated the progress already made in the field of tropical medicine, and discussed the vital contribution made by this specialty to the health and security of the United States and her allies during the recent World War. Emphasis was placed on the fact that the great tropical zone of the earth has always been a menace to world health. Since the beginning of human history, the tropics have constituted a vast reservoir for the unhampered breeding of innumerable diseases, many of which have previously invaded and established themselves in other regions. It was shown that within the brief span of a single lifetime tropical medicine has contributed enormously to the reduction of these so-called tropical diseases in certain temperate-zone countries, including the United States. As a consequence of the reduced incidence of such diseases since 1900, the medical profession of the United States gradually lost interest in tropical medicine until the beginning of World War II when we were faced with the danger of protecting our armed forces in the tropics. This threat to our military manpower created an intense new interest in tropical medicine which led to the development by the Allies of the most effective tropical-disease control program ever operated. In the United States, this program was carried out by the Army, Navy, and the Public Health Service with the assistance of every medical agency of the country. Its effectiveness is shown by the relatively low disease morbidity and mortality rates of our armed forces and by the fact that the country was protected against any serious invasion by disease.

¹ Presidential address, delivered at the dinner of the American Academy of Tropical Medicine, Miami, Florida, November 6, 1946.

² Brig. General, U. S. Army; Dean, Harvard School of Public Health.

THE FUTURE IMPORTANCE OF TROPICAL MEDICINE TO THE UNITED STATES

Tonight, I wish to talk about tropical medicine and the contribution it can make to global peace. Now that the war is over, the people of the world are actively concerned with the plans for a firm, lasting peace. The Axis forces surrendered more than a year ago; but international distrust and hatreds continue, and the fires of war still smolder and flare up in many places. The recurring wars of the past have been wasteful, destructive, stupid affairs, but they have been accepted as inevitable, and the human race had managed to blunder along in spite of them. Now, however, man is confronted by a dilemma. He is really worried about war. The super-explosions that levelled the Japanese cities of Hiroshima and Nagasaki last year have released a cloud of fear that hangs over the face of the earth—a chilling fear that war has become too dangerous for *Homo sapiens* and that man may not have sufficient intelligence to stop fighting and thus avoid his own destruction. Obviously, he must either control his elemental emotions sufficiently to work out some effective plan for enforcing peace or give up all pretence of superiority as a civilized member of the animal kingdom. Thus, we are faced with the challenge of establishing and maintaining world peace.

The American people are now confronted with the question as to how to meet this challenge of peace. The voices of the congenital “do-gooder” and the professional “Pollyanna” are again heard echoing the propaganda of foreign agents and fifth columnists who demand immediate disarmament. Any level-headed person knows that such talk is stupid and dangerous, because among nations, as among individuals, strength is essential to security. Therefore, the United States must maintain the armaments required for its protection and must continue to develop the health and strength of all its citizens. The advances already made in the nation’s health program must be extended, both for humanitarian purposes and because they are essential to national security. In this way, we shall insure our own strength and shall be better prepared to exert the sound leadership required to assist in the establishment and maintenance of a peaceful, civilized world.

Since tropical medicine still constitutes an important specialty in the field of medicine and public health, it must receive careful consideration in any plans for the improvement of national or international health. Its relative importance to the United States can be assessed by keeping in mind the problems to be faced and the measures required for their solution. The future problems naturally fall into two broad categories, namely, (1) the protection of Americans against tropical diseases at home, and (2) their protection against such diseases abroad.

CONTINUING PROBLEMS IN TROPICAL MEDICINE

In this country, as already stated, the endemic tropical diseases have been greatly reduced; but a number of infections are not yet adequately controlled. A variety of enteric infections, including amebiasis, the bacillary dysenteries, and the typhoid fevers, still occur in many parts of this country, especially in smaller communities. Murine typhus, Rocky Mountain spotted fever, epidemic

encephalitis, and relapsing fever occur in various sections. Sylvatic plague exists among rodents in wide areas of the West. Dengue fever is present in the South, where it frequently flares up in epidemic form. Finally, the great problem of malaria is still unsolved; and while its incidence is decreasing, it continues to take a heavy toll in some of the southern states. Therefore, it is important that full support be given to the United States Public Health Service and all other agencies now engaged in the control of these diseases.

Another important problem is presented by the need for developing more effective procedures to protect the United States against the future introduction and spread of tropical diseases. The emergency quarantine program developed jointly by the armed services and the United States Public Health Service during the war was effective under wartime conditions. Millions of men were brought home from tropical theaters without causing any serious epidemic of tropical disease. These men, however, were under strict military control and were subject to numerous examinations and inspections which started before they left their tropical stations and continued until their discharge from service in this country. Also, special precautions were taken to sanitize and to enforce insect and rodent control at all military installations, including hospitals in the United States, and thus minimize the spread of infection to the civil population. The quarantine program of the future must be geared to meet the somewhat different peacetime civilian conditions and requirements. It will be concerned largely with rapid civilian travel by air and water, and some of the safeguards employed during the war will not be applicable. Air travel is now so rapid that it is possible for a traveler in any part of the tropical zone to contract a serious disease, board a fast plane, and debark in an American city before the end of the incubation period. Therefore, it will be difficult to exclude all diseases by quarantine alone, and this procedure must be supplemented by an alert medical profession, including practitioners and health officers who are sufficiently well trained in tropical medicine to identify and prevent the spread of such infections as may be introduced.

Tropical medicine will also be of continuing importance to this country because of the increasing numbers of Americans who will go to tropical countries either as tourists or residents for social, industrial, or military reasons. World travelers will need advice as to how to protect themselves against cholera in India or against sleeping sickness in Liberia or scrub typhus in New Guinea. Diplomats, missionaries, and others residing in tropical countries will need protection against local diseases. American troops stationed in overseas tropical garrisons will require effective control programs to maintain their health, especially during maneuvers and periods of field service. Finally, while we hope for a lasting peace, we must be prepared for war in the tropics; and we must continue to search for better methods for the treatment of tropical diseases and for their prevention under military conditions.

Thus, our tropical medicine problems are not localized in the continental United States but extend to many foreign countries, and it is obvious that we must carry the attack to the tropical zone. In the past, this has been done on a

limited scale by certain industrial organizations operating in tropical countries, in order to protect their employees and to increase production. Co-operative health work has also been sponsored by the Pan-American Sanitary Bureau, the International Health Division of the Rockefeller Foundation, and other large organizations. During the war, an extensive health program was operated by tropical countries of the western hemisphere with the co-operation of the office of the Co-Ordinator of Inter-American Affairs. This international attack on the diseases of the tropics should become more comprehensive and effective under the operation of the World Health Organization.

PLANS FOR THE FUTURE

From the foregoing summary of the remaining problems in tropical medicine, it is obvious that this subject is still of vital importance to American medicine and to world health. We are now faced with the question as to what must be done to meet these problems. The answer is suggested by the manner in which the nation met the emergency situation during the war. The wartime program consisted of four major parts: (1) the collection of current information about disease hazards throughout the world and the dissemination of this information to all concerned; (2) the improvement of teaching in tropical medicine in civilian medical schools and schools of public health; (3) the development of a comprehensive national program of medical research to search for new knowledge about the etiology, diagnosis, treatment, and prevention of tropical diseases; and (4) the establishment of the operating agencies required to apply all of the available knowledge in the protection of our citizens and the members of our armed forces.

The leadership furnished by the American Academy and the American Society of Tropical Medicine contributed much to the success of the wartime program. These two organizations now have an opportunity to continue their leadership. The peacetime attack on tropical disease has already started, but it must be organized, expanded, and carried forward with energy if it is to be completely effective.

Medical Intelligence. The collection of current information on the world prevalence of disease is now being carried out by various agencies, including the Public Health Service, the Army, and the Navy.

Teaching. Interest in the teaching of tropical medicine in medical schools and schools of public health increased during the war; but there is still room for improvement. In 1941, a Committee on the Teaching of Tropical Medicine was appointed by the Association of American Medical Colleges to determine the status of the teaching of tropical medicine in the medical schools of the United States and Canada, and to make recommendations to meet the wartime emergency. This Committee consisted of Doctors Meleney, Soule, and Kostmayer. Their report, made in July, 1946, mentions the valuable assistance rendered by Mr. Archie Woods of the John and Mary Markle Foundation, which appropriated a large sum of money to support a program for the intensive instruction in tropical medicine of teachers in civilian medical schools. This program was requested by the Preventive Medicine Service of the Army because a large proportion of

the young doctors entering military service had not received adequate training in the subject and there was not time to train them after they were commissioned. To meet this situation, the Markle Foundation made available through the National Research Council, sufficient funds to enable two instructors from each civilian medical school in this country and in Canada to attend the eight-weeks' courses in tropical medicine given at the Army Medical School or at Tulane. Later, the Markle Foundation provided additional funds to enable these instructors to take additional field training in Central America. A total of 212 such fellowships were awarded, of which 110 were for courses in this country and 102 for training in Central America.

As shown in Dr. Meleney's report, various other important educational activities were started either by the Committee, by the National Research Council, or by other agencies. Through the National Research Council, arrangements were made for travelling lecturers to speak on tropical diseases in medical schools. The Army Medical School and the Army Medical Museum established centers for the distribution of specimens to be used in teaching the parasitology and the pathology of tropical diseases. Arrangements were made to prepare and distribute pertinent articles and books to civilian medical schools. In 1944, a Committee on War and Post-War Tropical Medicine, appointed by the Society, recommended to the United States Public Health Service that they establish a diagnostic laboratory for tropical diseases and an information service, in the Office of Malaria Control in War Areas, Atlanta, Georgia. This laboratory, which now operates as the Communicable Disease Center in Atlanta, trains technicians in parasitology and tropical medicine and prepares lantern slides, film strips and motion pictures for local use and for loan to teaching institutions. The civilian schools have also been assisted by the American Foundation of Tropical Medicine and other agencies through the grants used to pay the salaries of instructors in tropical medicine and for the support of research.

Dr. Meleney's Committee, in its final report, concluded that there had been an increase in instructors in parasitology in the medical schools of the United States and Canada. An analysis of questionnaires sent to eighty-one schools in August, 1945, showed that the total number of instructors had increased from 155, prior to the war, to 206 in 1945. In the four-year schools, the instructors in tropical medicine had increased from a pre-war figure of 49 to 151. Sixty-nine of the schools indicated that they would like to continue the expanded wartime programs of tropical medicine and parasitology, but most of them stated that to do so they would need additional teaching staff, which would require outside financial assistance. The report of this important Committee indicates an increased interest in the teaching of tropical medicine in American medical schools.

It is hoped that the schools can find the necessary financial support and that, in the near future, tropical medicine will be adequately covered as a normal part of American medical education. The Academy and the Society can make a lasting contribution to our national health by sponsoring this important educational program.

Research. The success of these plans for the future will depend, not only on

the development of better teaching in tropical medicine, but on the continuation of a broad program of research. In spite of the progress already made, there are still many gaps in our knowledge of the diseases of the tropics. The necessity of continuing research in this field is obvious when one considers the contribution which it has made to public health within the relatively short period since the Civil War. The basic discoveries made in protozoology, bacteriology, virology, and medical entomology afforded information concerning the cause, transmission, treatment, and prevention of a host of tropical diseases. Application of this information contributed much to the rapid improvement of public health in this and other temperate-zone countries during the present century. Likewise, the great national research program, organized by our government to meet the emergency conditions of World War II, afforded new agents and methods for the attack on tropical infections. Various aspects of this program were carried out by the Army, Navy, the Public Health Service, and many other governmental and non-governmental agencies. They were co-ordinated and implemented to meet the nation's wartime needs through the National Research Council and the Committee on Medical Research.

The entire medical resources of the country were utilized in this great effort. The cost of the program was high, but the investment paid rich dividends by providing new and effective agents and methods for the fight against tropical diseases. As a consequence of this program, we no longer fear epidemic typhus fever, for we know that we can prevent the spread of this ancient scourge by the wholesale use of vaccines and DDT louse powder. We also have new methods for the prevention of epidemics of plague in man. More effective and more economical methods are now available for use against other insect-borne diseases, including murine typhus, scrub typhus, yellow fever, dengue, and malaria. New chemotherapeutic agents are available for the use of many infections formerly considered dangerous, and new fundamental information is at hand which will undoubtedly lead to even more important discoveries about tropical diseases in the future.

The success of this National research program was apparent to many of us long before the end of the war. Consequently, strenuous efforts were made to insure the continuation of such a program in the postwar period. Unfortunately, the Committee on Medical Research was a temporary agency set up by the President under the Office of Scientific Research and Development only for the period of the emergency. It was, therefore, proposed that a new organization, to be known as the National Science Foundation, be established to take its place. As you know, there has been much discussion about this Foundation. Numerous congressional bills have been proposed, but none of these bills have passed. It is hoped that the Academy will help in the development of an adequate national program of medical research which will include all phases of research on tropical medicine.

In the meantime, much of the research started during and before the war is being conducted by various agencies of the government, including the Army, Navy, the Public Health Service, the Veterans Administration, the Department

of Agriculture, and by others including; the medical schools; and the philanthropic foundations.

INTERNATIONAL OPERATIONS

Because of the international importance of tropical diseases, those concerned with their control have always realized the necessity of extending their activities into the tropical zone. For many years, workers in the United States have been interested in the improvement of health conditions outside our continental limits. That this interest is mounting is evidenced by the number of agencies that are now operating in tropical places or are planning to do so. For example, the Army and Navy are planning to continue and expand many of their pre-war research and control activities in Latin America, the Pacific and elsewhere. The Pan-American Sanitary Bureau and the Office of the Co-Ordinator of Inter-American Affairs still have extensive health programs in co-operation with the Latin-American countries. The Gorgas Memorial Laboratory is still active in Panama. The American Foundation of Tropical Medicine is now planning to establish a tropical medicine research institute in Liberia. The International Health Division of the Rockefeller Foundation is continuing and expanding its great field work with tropical diseases in many parts of the world. Certain of our universities, including Columbia and Cornell, have established connections with medical schools in the American tropics, and plans are being made by various organizations to increase the exchange of trained workers and faculty members between the United States and various tropical countries. Undoubtedly, similar operations are being planned in many other countries. Another indication of the rising tide of interest in research on an international basis is afforded by the Pacific Science Conference, which met last June in Washington, D. C., at the call of the National Research Council. It was the purpose of this conference to form an effective organization of scientists, interested in the Pacific area, to encourage and assist scientific research and activities in the Pacific area and to further international co-operation along these lines. Its division devoted to public health and medicine has drawn up recommendations for a broad program of research on the disease problems peculiar to Americans who visit or reside in the Pacific area and to native populations.

This interest in research must be fostered, supported, and directed toward the solution of our still unsolved health problems.

Further evidence of the increasing interest in the international aspect of tropical medicine is afforded by the current plans initiated by the Academy and the Society, to hold the Fourth National Congress on Tropical Medicine and Malariology in this country during 1947. Preliminary plans for the Congress have been made with the State Department by Dr. Mark Boyd. This Congress should afford a common meeting place at which the ideas and objectives of our American group can be integrated with those of similar groups from other countries in order to work out a sound program for the international attack on tropical disease.

WORLD HEALTH ORGANIZATION

The timing of this proposed Congress is fortunate, for it comes at a time when real progress is being made in the establishment of the World Health Organization. You will recall that the International Health Conference was the first meeting to be called by the United Nations Organization and was the first conference to which neutral states were invited as observers. On July 22, this year, representatives of sixty-one nations signed the constitution of this, the first fully empowered international agency in public health. The final act of this conference included approval of the constitution of the World Health Organization.

The preamble to the constitution is as follows:

"Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

"The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition.

"The health of all peoples is fundamental to the attainment of peace and security and dependent upon the fullest co-operation of individuals and States.

"The achievement of any State in the promotion and protection of health is of value to all.

"Unequal development in different countries in the promotion of health and control of disease, especially communicable disease, is a common danger.

"Healthy development of the child is of basic importance; the ability to live harmoniously in a changing total environment is essential to such development.

"The extension to all peoples of the benefits of medical, psychological and related knowledge is essential to the fullest attainment of health.

"Informed opinion and active co-operation on the part of the public are of the utmost importance in the improvement of the health of the people.

"Governments have a responsibility for the health of their peoples which can be fulfilled only by the provision of adequate health and social measures."

The objective of the World Health Organization is the attainment by all peoples of the highest possible level of health. In a recent article, Shimkin outlined the functions of the Organization and commented as follows:

"The first tasks of the World Health Organization undoubtedly will concern themselves with the age-old scourges of man, accentuated by the devastation of the war. The need is urgent for caring for the sick and wounded, for feeding the hungry, controlling epidemic diseases, and providing basic environmental sanitation. By pooling the resources, knowledge, and skills of all nations, elimination of such diseases as malaria, tuberculosis, and syphilis can be achieved.

"Beyond these immediate needs, the World Health Organization looks forward toward leading the struggle in each country, with the help and encouragement of all other countries, of long-term programs of health services to protect the people from ravages of disease and to insure to every individual a standard of health compatible with the technical achievements of the medical sciences. And, using the broad definition of health, the goal of application of technical achievements to all men is not limited to physical well-being. Mental hygiene, in helping man

to adjust to his environment, must be used in combination with education to prevent the insanity of another total war and to destroy the basic causes of war."

On closing the Conference on July 22, its Chairman, Dr. Thomas Parran, made the following remark:

"The World Health Organization is a collective instrument which will promote physical and mental vigor, prevent and control disease, expand scientific health knowledge, and contribute to the harmony of human relations. In short, it is a powerful instrument forged for peace."

The progress made toward the development of this World Health Organization is heartening to all of us. It has a special significance to those of us who are concerned with tropical medicine. As indicated in this talk, the diseases of the tropics still constitute the major hazard to world health; and the control of such diseases must, of necessity, become one of the major problems confronting the World Health Organization. I am sure that the members of this Academy, like every other organization concerned with tropical medicine, are looking forward with eagerness to accepting this challenge of the future.

THE DISTRIBUTION OF EXOERYTHROCYTIC PARASITES AND THE TISSUE REACTION CAUSED BY BLOOD-INDUCED *PLASMODIUM GALLINACEUM* IN CHICKS^{1, 2}

JOHN L. TULLIS³

INTRODUCTION

One of the more interesting features concerning the asexual life cycle of *Plasmodium gallinaceum* is the presence of exoerythrocytic, non-pigmented schizonts. James and Tate (1) discovered them in 1937 and their findings were confirmed shortly after by Brumpt (2) who had originally isolated the parasites from the erythrocytes of chickens in 1935 (3). James and Tate (4, 5) expanding their studies were able to observe non-pigmented schizonts in the reticulo-endothelial⁴ cells of the liver, spleen, kidneys, brain and other internal organs and in circulating leukocytes.

James (6) found the exoerythrocytic schizonts were equally common in chicks under suppressive quinine therapy after both sporozoite-induced and blood-induced infections. In the untreated disease in the blood-induced cases, the exoerythrocytic forms were rarely observed, but in the sporozoite-induced cases they were numerous.

The experiments described below were designed to (a) determine the distribution of exoerythrocytic parasites in blood-induced *Plasmodium gallinaceum* infection in untreated and quinine-treated chicks and to (b) determine the tissue reaction to the infection. Study of the brain will be the subject of a second report.

METHODS AND MATERIALS

Parasites. The 8A strain of *Plasmodium gallinaceum* was used. This is a portion of the original strain isolated by Brumpt "chez la poule domestique" in Ceylon in 1935. The strain was sent to Dr. Beltran at the Institute of Hygiene and Tropical Diseases in Mexico, from there to the Rockefeller Institute, and from there to the National Institute of Health from which it was obtained by this Institute.

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² The material in this article should be construed only as the personal opinion of the writer and not as representing the opinion of the Navy Department officially.

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⁴ The term "reticulo-endothelial cell" was used by James and Tate to include both fixed non-phagocytic cells lining blood vessels (true endothelial cells) and the widely dispersed group of phagocytic cells which (a) line the sinusoids of the liver, spleen, and bone marrow and (b) are found in connective tissue in both resting and wandering states. Individual cells in the latter group are known as macrophages, histiocytes, phagocytes, reticulo-endothelial cells, polyblasts, adventitial cells, clasmatoocytes, endotheliocytes, wandering cells and transitional cells, and in the liver, are known also as Kupffer cells. This report adheres to a distinction between true endothelial cells and reticulo-endothelial cells.

Hosts. Five-day old laboratory-bred Rhode Island Red chicks weighing approximately 50 gm. each were used. The disease was transmitted by the injection of 16×10^6 infected red blood cells into the wing vein. The experimental animals were divided into two groups. The first group received no treatment while the second was treated with daily oral doses of quinine hydrochloride (70 mg./kg. of body weight) by means of stomach tube beginning 24 hours after inoculation with the parasite in order to suppress the erythrocytic cycle.

Clinical evidence of infection is abrupt in its onset. A chick that appears active and normal may within 24 hours become lethargic and weak. It loses muscle tone rapidly, the eyes become dull, the eyelids remain partially closed, the flight reflex is lost, the appetite is poor and the bird is likely to die. The suddenness of this change and the rapid downhill progress once the change has taken place is emphasized.

Four normal chicks and four chicks which had received 11 to 14 doses of quinine, but were otherwise normal, were used as controls.

Chicks were sacrificed and autopsies performed between 2:00 and 4:00 P.M. in group 1, and the majority in group 2 were sacrificed during the same period.

Histological methods. Thin blocks of the various organs were placed in formol-Zenker solution for 12-24 hours, washed for 12 hours, and transferred to 80 per cent alcohol. They were then embedded in celloidin, serially sectioned at 8 micra and stained with hematoxylin-eosin-azure (HEA). The technic used in preparation of the slides and the terminology used in description of the cells closely follow the methods and terminology of Maximow and Bloom. HEA stains the parasites various shades of blue, the distinctive red of the chromatin brought out by the Giemsa stain being absent. The parasites are fairly easily identified, particularly after experience is acquired, and what is lost by the lack of distinctive chromatin stain is made up for by the excellence of the differentiation of the host cell. Malaria pigment appears as dark brown irregular clumps of varying size.

RESULTS

The pathogenicity of blood-induced *Plasmodium gallinaceum* infection in chicks is high. With an inoculation of 16×10^6 parasites, all 30 chicks which did not receive suppressive therapy either died or showed marked clinical evidence of acute malaria on or before the seventh day post inoculation. Sixteen chicks were not autopsied; the remaining 14 are described below.

From the second to the seventh days, pigment-filled macrophages and parasitized erythrocytes are seen in all the chicks. Early in the infection, both types of cells are widely dispersed and few in number. Their numbers increase as the disease progresses.

In the spleen there is a relative absence of pigment in the macrophages of the white pulp as compared with the amount in the macrophages in the red pulp, particularly those in close proximity with the sinusoids. Elsewhere, malaria pigment is concentrated where the macrophages are most abundant, such as in the reticulo-endothelial cells lining the sinusoids of the liver, spleen and bone

marrow and in the tissue (extravascular) macrophages of the lungs and intestines.

The parasitized erythrocytes are observed in the blood vessels of all organs. Some of the red cells contain two parasites which cause slight swelling of the cell and peripheral displacement of the nucleus.

Exoerythrocytic parasites first appear on the fourth day after inoculation in the form of non-pigmented schizonts in rare circulating macrophages and occasional reticulo-endothelial cells in the white pulp of the spleen. The lack of pigment is characteristic of exoerythrocytic parasites.

The number of merozoites in the schizonts in reticulo-endothelial cells varies from 20 to 80. The schizonts in circulating macrophages usually contain only 6 to 8 merozoites. The schizonts are spherical to ovoid in shape and average, in reticulo-endothelial cells, 12 to 14 micra in diameter. The merozoites in any one schizont are similar, but may vary in shape from spherical to ovoid in different schizonts. The peripheral merozoites are usually arranged in an orderly rosette pattern and are fairly evenly spaced. Between the peripheral merozoites and the "retaining membrane"⁵ of the schizont, the cytoplasm of the parasite is represented as a narrow clear halo, a variable portion of which may be artefact.

The large size of the parasite in the reticulo-endothelial cells causes distortion of the host cell, frequently to the extent of obscuring both cytoplasm and nucleus. Such parasites appear as if they were free in the lumina of blood vessels and sinusoids. Where the schizont consists of fewer merozoites and the section is through the level of the nucleus of the host cell, the nucleus is flanked by small portions of cytoplasm and the cell membrane can be traced to a point where it blends with the "retaining membrane" of the schizont.

On the sixth and seventh days, the exoerythrocytic schizonts are found fairly frequently in the reticulo-endothelial cells of the spleen (both red and white pulp) (plate 1, fig. 1) and liver, and in the endothelial cells lining the blood vessels of the heart and lungs. A rare parasite is seen in reticulo-endothelial cells of the lamina propria of the intestine. Exoerythrocytic schizonts are not found in the kidneys, bone marrow, striated muscle or pancreas. Throughout the acute stage of the disease, erythrocytic trophozoites and schizonts far exceed the number of exoerythrocytic schizonts. No exoerythrocytic trophozoites are seen.

Twenty-nine of 30 chicks treated with quinine remained healthy and had no clinical or pathological evidence of malaria until the twelfth day post inoculation when there was a sudden and dramatic change.

In contrast to the findings in the acute stage of the disease, the tissues of the chicks on and after the twelfth day post inoculation contain only rare erythrocytic parasites, while the exoerythrocytic parasites are abundant. Non-pigmented schizonts appear commonly in the reticulo-endothelial cells of the liver and spleen, and in the endothelial cells of the blood vessels of the heart and lungs, less frequently in the intestine, pancreas, fat and kidneys; and only occasionally in the bone marrow and striated muscle. The non-pigmented schizonts

⁵ The existence of such a membrane is debatable, but the term is a useful one.

appear identical with those described in the acute stage (plate 1, figs. 2, 3 and 4).

Seventeen chicks, given suppressive quinine therapy, were not killed until they showed some clinical evidence of infection. One hour before autopsy they received an intravenous inoculation of 0.25 cc. 25 per cent Higgins India ink in order to accentuate the reticulo-endothelial system. One was discarded as a technical failure.

The autopsies on the ninth, tenth and eleventh days post inoculation proved to be premature for no parasites are present in either blood stream or tissues. This would indicate that the chicks may become sick before the parasite is demonstrable in either the tissues or blood stream. However, from the twelfth to the twenty-fifth day, exoerythrocytic schizonts are abundant. Their appearance does not differ from that of the reticulo-endothelial parasites described above (plate 1, figs. 5 and 6). The order of frequency of the appearance of the parasites in the organs is only slightly different from the group not injected with ink. Parasitized erythrocytes and circulating macrophages are not prevalent, but both may be seen occasionally.

Plasmodium gallinaceum does not cause a marked tissue reaction. The slate gray color of the liver and spleen in the acute stage of the disease is due to deposits of malaria pigment in the macrophages. There may be slight darkening of the lungs and bone marrow for the same reason, but the reaction is never as intense as in the liver and spleen. The other organs show no color change. In the chronic stage, erythrocytic parasites are so few and, therefore, pigment is so scanty, that color changes are usually not observed in any organ.

The liver and spleen show varying degrees of enlargement in both stages of the disease. The enlargement depends chiefly on distention and engorgement of the sinusoids. Proliferation of the reticulo-endothelial system is debatable, but at best is not marked and does not account for liver or spleen enlargement. Although exoerythrocytic schizonts may enlarge to such an extent that they appear to occlude capillaries, no infarction results. In one very heavily parasitized chick in the chronic stage of the disease, small foci of liver cell necrosis are seen. This cannot be explained on a basis of vascular change, but resembles a toxic effect due to overwhelming infection.

Small hematopoietic centers in the liver are more frequently seen in infected chicks on and after the fourteenth day post inoculation than in the controls. The bone marrow does not show a corresponding hyperactivity. The chicks are growing animals so that active hematopoiesis is the rule, but there is no marked difference in the marrow of infected and normal control chicks.

One very interesting feature is the fairly consistent change in the liver cells in the patent phase of the disease in quinine treated chicks. The normals, the quinine controls, the acutely infected and the chicks in the prepatent phase of the quinine treated infection all possess similar liver cells. They are large cells with indistinct margins. Their nuclei are large, ovoid, and deeply staining. Their cytoplasm is clear and reticulated, the type usually associated with high glycogen content and denoting a well-fed animal. By contrast, the liver cells of the quinine-treated chicks with patent malaria are, in a high percentage of cases, small and often sharply outlined. Their nuclei are smaller, but they stain

normally. Their cytoplasm has a solid or granular appearance and the deep stain indicates a relative absence of glycogen (plate 2, figs. 7, 8, and 9).

DISCUSSION

The existence of exoerythrocytic schizogony and the reaction of exoerythrocytic parasites in the presence of therapeutic agents is of great importance for a complete understanding of *Plasmodium gallinaceum* malaria. It is evident that quinine hydrochloride in large oral doses is capable of suppressing erythrocytic schizogony but has no such action on exoerythrocytic schizogony. The lives of infected chicks are prolonged by quinine therapy, but the disease is not cured.

Huff and Coulston (7) and Coulston *et al.* (8) have traced the early development of *Plasmodium gallinaceum* from sporozoite through exoerythrocytic schizogony to erythrocytic trophozoite. They have proved that sporozoite-induced malaria gains its first foothold in the reticulo-endothelial cells of the connective tissue. It has been pointed out above that on and after the fourth day of blood-induced malaria, parasites in widely distributed reticulo-endothelial cells are present along with erythrocytic parasites. Thus, it can be concluded that in both sporozoite-induced and blood-induced *Plasmodium gallinaceum* malaria, the parasite finds a suitable habitat in reticulo-endothelial cells. In these cells development can progress in spite of the presence of suppressive levels of quinine in the blood stream.

Morphologically there is nothing to distinguish erythrocytic merozoites from exoerythrocytic merozoites. However, a metabolic difference in the two is suggested by the loss of liver glycogen in the quinine-treated chicks with patent malaria. The loss of liver glycogen may, on the other hand, be due solely to anorexia resulting from toxicity of the infection. The disparate response to quinine therapy suggests a physiologic dissimilarity in the parasites and/or their host cells. It is possible that the endothelial and reticulo-endothelial cells act as a barrier to quinine, thereby prohibiting contact of the quinine and the parasites in these cells or an agent capable of neutralizing quinine may be present in endothelial and reticulo-endothelial cells. Once the life cycle is established, it is only through a fuller understanding of the physiology of the exoerythrocytic parasite and its host cell that a rational approach to the therapy of malaria can be made.

SUMMARY

1. Untreated *Plasmodium gallinaceum* infection in chicks is an acute disease characterized by large numbers of pigment-producing parasites in erythrocytes and only a few non-pigmented parasites in reticulo-endothelial cells of the spleen, liver and intestine, and in the endothelial cells lining the blood vessels of the heart and lungs.

2. On the other hand, the disease in chicks receiving quinine in suppressive doses is chronic and is characterized by large numbers of non-pigmented schizonts in the reticulo-endothelial and the endothelial cells of all the organs examined and only rare parasitized erythrocytes.

3. It appears that the metabolic requirements of erythrocytic and exo-

erythrocytic parasites may be different because the two forms of the parasite may provoke varying reactions in the liver and because they respond differently to quinine therapy. The glycogen depletion in the liver cells in quinine-treated chicks may, however, be a function of the general toxicity of the disease rather than of the metabolic requirements of the exoerythrocytic parasites.

ACKNOWLEDGMENTS

The author wishes to express his indebtedness to Lieutenant Isidore Gersh H(S), USNR and to Lieutenant Commander Levon A. Terzian, H(S) USNR for their many helpful suggestions.

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PLATE 1

PHOTOMICROGRAPHS OF EXOERYTHROCYTIC SCHIZONTS OF *PLASMODIUM GALLINACEUM* (ARROWS)

FIG. 1. A non-pigmented schizont in a tissue macrophage in the white pulp of the spleen six days post inoculation is illustrated. The host cell nucleus is out of focus. The cell membrane is poorly outlined. The two larger black bodies in the vicinity of the parasite are artefacts. $\times 2000$.

FIG. 2. A reticulo-endothelial cell in the white pulp of the spleen 12 days post inoculation contains a large spherical parasite. The host cell is not well defined. $\times 2000$.

FIG. 3. Two non-pigmented schizonts are visualized in an alveolar septum of the lung 12 days post inoculation. The reticulo-endothelial host cells are not in focus. $\times 2000$.

FIG. 4. A venule in the lung 12 days post inoculation contains a parasitized endothelial cell. Note the large size of the parasite compared with the erythrocytes in the lumen of the vessel. $\times 2000$.

FIG. 5. An endothelial cell lining a capillary in the heart contains a non-pigmented schizont on the twenty-fourth day post inoculation. The nucleus and the membrane of the host cell are fairly well seen. $\times 2000$.

FIG. 6. A non-pigmented schizont is shown in an endothelial cell in a capillary of the myocardium 24 days post inoculation. The nucleus of the host cell is above and to the left of the parasite. $\times 2000$.

PLATE 1

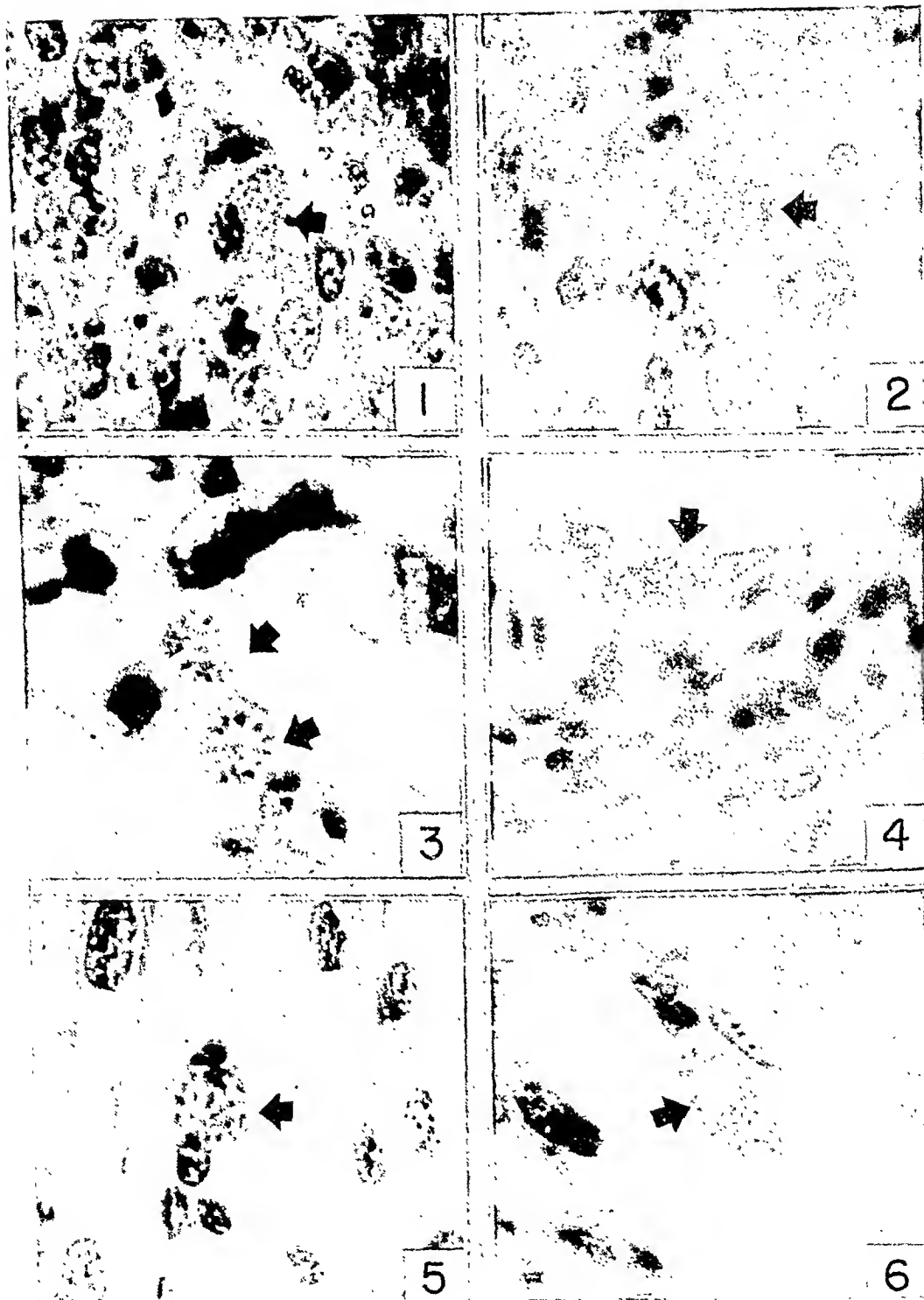


PLATE 2

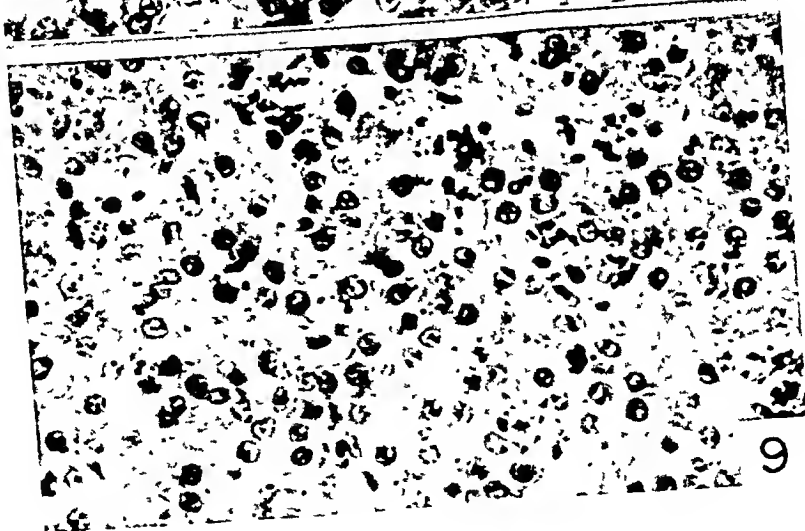
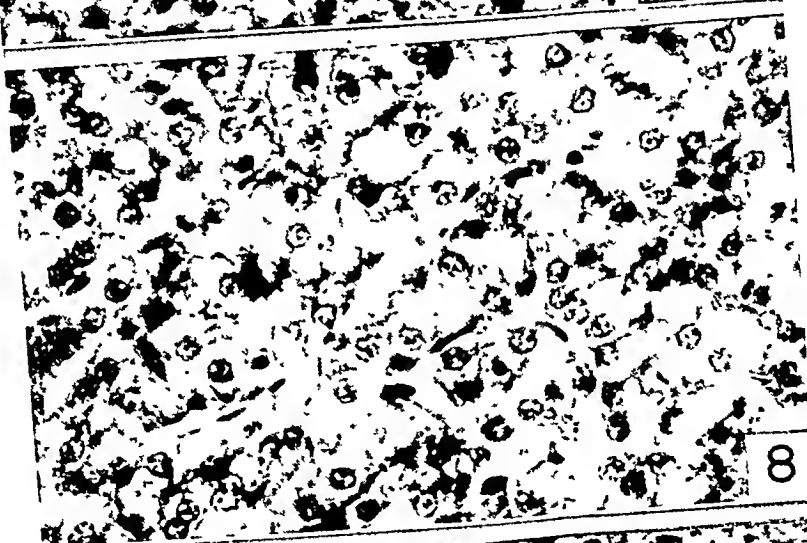
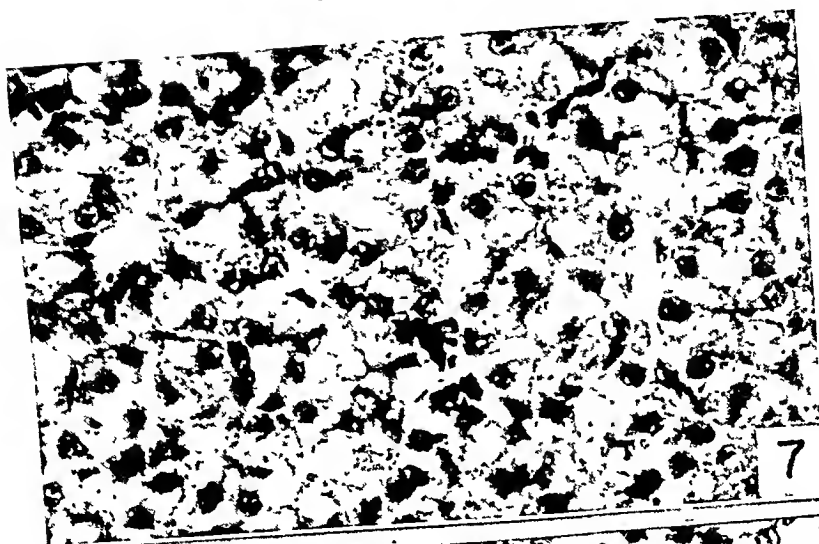
PHOTOMICROGRAPHS OF THE LIVER IN ONE NORMAL AND TWO MALARIOUS CHICKS

FIG. 7. Liver in a control animal injected with India ink. Note the ink granules in the Kupffer cells. $\times 400$.

FIG. 8. Liver 10 days post inoculation in a chick receiving suppressive quinine therapy. Note the large, glycogen-filled cells and general similarity to the control liver. $\times 400$.

FIG. 9. Liver 24 days post inoculation in a chick receiving suppressive quinine therapy which had, within a few hours, developed signs of malaria. The tissues contain numerous exoerythrocytic forms (not shown here). The cells in contrast to those in figures 7 and 8 are smaller and shrunken. Their cytoplasm is solid or granular and their glycogen content is depleted. The black globules are masses of ink in the Kupffer cells. $\times 400$.

PLATE 2



STUDIES ON ATABRINE (QUINACRINE) SUPPRESSION OF MALARIA

III. THE EPIDEMIOLOGICAL SIGNIFICANCE OF ATABRINE SUPPRESSION

F. B. BANG¹ AND N. G. HAIRSTON²

The campaigns in the South and Southwest Pacific were the first in which a fighting army remained at length in a hyperendemic malarious area. Without the adequate mass suppression of clinical malaria afforded by atabrine, these campaigns would not have been possible. Overall suppression in a large body of men has been found more effective epidemically than the sum of suppressed individual attacks. An understanding of the mechanism of suppression and its dampening effect on epidemic malaria is attempted in this paper.

Atabrine not only kills vivax gametocytes but prevents the development of both vivax and falciparum gametocytes in adequately suppressed individuals. Thus it prevents the infection of mosquitoes which in turn means fewer infections of other soldiers. This makes it possible for troops not only to be protected during their stay in malarious areas but results in a much lower residual infection.

Epidemic falciparum malaria in the tropics acquires its explosive nature by a geometric increase in the number of crescent carriers in the non-immune population (1, 2). Although the increase of anophelines brought about by the troop operations also plays a large part in the genesis of the epidemic, an increase of anophelines alone with a minimal number of gametocyte carriers is incapable of producing the epidemics experienced by both the American and Australian Armies in the early campaigns.

The combination of (a) an efficient vector population (b) many susceptibles, and (c) a few infections to start the chain of transmission makes epidemic malaria in the absence of suppression inescapable. This has been true regardless of the size of the original reservoir. One of the most devastating military epidemics occurred during the Civil War in the Southern United States (3) where, compared with New Guinea, malaria is only moderately endemic. So the amount of infection in the natives is of relatively little importance in outbreaks of epidemic malaria in unprotected troops. This was particularly true in New Guinea where contact with the natives was slight. Infection of mosquitoes by the enemy troops previously occupying an area has a slight and short lived effect.

INFECTION RATES IN NEW GUINEA³

Although the conditions leading to epidemic malaria are recognized, no adequate studies of the gametocyte rates and mosquito infection rates from the

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³ Study of gametocyte rates in humans and their relation to infection rates in mosquitoes in New Guinea may be simplified by considering the vector anopheline as one species. This point is debated by taxonomists, but since the two or three races are so similar in feeding habits and ability to transmit malaria (4), we have treated them as one.

beginning to the end of an epidemic are available in the literature. The best we can do is to compare the infection rates in endemic and epidemic situations about which we have sufficient information.

The lowest reported infection rate (0.4 per cent of 2522 salivary gland dissections) was obtained in a native village near Lalapipi, where malaria is hyperendemic but where there are no susceptible non-immunes (5).

Heydon's studies at Rabaul in and around a hyperendemic village showed a sporozoite rate of 3.9 per cent and an oocyst rate of 3.6 per cent in 220 dissections (6), despite a low parasite rate in the natives. He pointed out, however, that since most of the infected anophelines were collected from houses with known gametocyte carriers and since they were kept for some days before dissection this rate does not reflect the general rate in nature (7).

The outstanding epidemic of malaria in an unprotected and non-immune population in New Guinea occurred in the Netherlands East Indies colony of Tanah merah (8). Here the attack rate reached an average of four yearly attacks per person. It was during this epidemic that the highest infection rate of *A. punctulatus (moluccensis)* was obtained, 12.7 per cent (63 dissections). A lower rate is recorded before and after this peak (9) but dissections were not numerous enough to be statistically significant. Other records of dissections are available (4) but there is not sufficient information about the amount of malaria present.

DATA FROM TROOPS

Gametocyte rates and infection rates of mosquitoes caught in troop areas are presented in Tables I and II.

Since atabrine has a direct effect on the vivax gametocytes, but none on the falciparum crescents, we may expect, and do find, that with good but imperfect suppression only falciparum carriers are discoverable in the troops.

We conclude from the above that most infections acquired by troops under poor or inadequate suppression come from the troops themselves, and as suppression becomes more perfect a larger percentage of the infections come from the natives and enemy troops.

STUDIES ON SPECIFIC UNITS (INFANTRY TROOPS)

Two fairly detailed studies on sections of two divisions having contrasting experiences are presented as illustrations of the efficacy of good atabrine discipline in preventing the infection of large numbers of men even though they fought in hyperendemic areas.

A Division. The malaria rates for a regiment of a fresh USA Division were as shown in Table III (previously during staging they had had only a few cases in the whole division).

Surveys were conducted on the troops on the 24th April and 13th July, 1½ and 4½ months respectively after the landing on the Admiralty Islands. On the first survey atabrine levels were very satisfactory (see below) and 2.2 per cent of 184 men showed positive smears. On the second survey the atabrine levels

had dropped so much that had this group of men been infected, a high malaria rate would be expected. Actually, as shown above, they did not have a high rate and only 3 per cent of 200 men showed positive smears.

These results were aided by the fact that Los Negros, the first island to be invaded, is a predominately coral island, so neither adult nor larval anophelines were common. Only 30 adults were taken on repeated collections near the native

TABLE I
Gametocyte rate in troops

ORGANIZATION	MAL. RATE PER 1000 MEN/YEAR	NO. MEN EXAMINED	GAMETOCYTE RATE
X Brig.*.....	7400	90	10.0 per cent P.f. 8.0 per cent P.v.
Y Aus. Fld. Coy.*.....	3360	88	3.4 per cent P.f. 9.2 per cent P.v.
Mixed Troops 1944.....	200	2000	0.25 per cent P.f.

* We have used these figures on Australian troops, since during the period of scanty suppression, no figures are available on the American troops having similar malaria rates.

TABLE II
Mosquito dissections in troop areas

LOCATION	MALARIA RATE/1000 MEN/YR.	NO. MOSQ. EXAMINED	POSITIVE		RATE <i>per cent</i>
			Guts	Glands	
Ramu Valley, Dec. 3-17 (10)...	4060	154	4	3	4.5
Jan. 7.	2440	139	1	2	2.2
Scattered troop areas 1944	200	677	2	1	0.5

TABLE III

MONTH	RATE (1000 MEN) YEAR
March... ..	48
April	50
May	48
June	48
July... ..	50

village. The paucity of anophelines on Los Negros cannot, however, explain the overall low rate present in the whole division throughout the period of combat and following, for anopheline larvae and adults were very common on the muddy clay soil of Manus Island. One evening's all-night catch in an ANOMALIA camp about a week after combat troops had left the area yielded over 1500 mosquitos. We suggest then that the campaign on the Admiralty Islands was successful

example of the ability of atabrine suppression to prevent epidemic malaria (and thus a large number of infections), even during combat in a hyperendemic malarious area.

B Division. A different picture is presented by B Division. We did not follow the same regiment through both the Saidor and Aitape Campaigns, but since its three regiments had similar experiences with malaria in these two campaigns, we will present the data on I Regiment at Saidor, and for the II Infantry Regiment at Aitape as illustrative of the Division (Table IV).

Previous campaigns had left them with a large load of infection to start

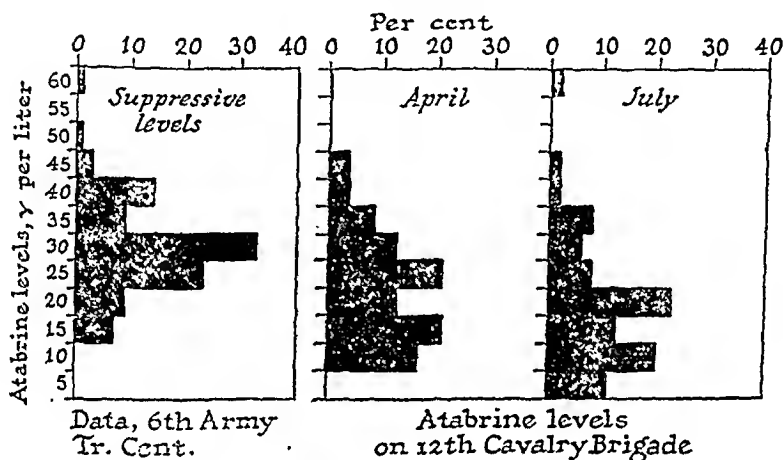


FIG. 1. ATABRINE LEVELS ON A DIVISION

TABLE IV
Malaria rates per 1000 per year

MONTH	I	II
January.....	44.7	603.7
February.....	88.4	174.7
March.....	175	263.5
April.....	184	199
May.....	90	294
June.....	222	371
July.....	150	134

with, although by the end of 1943 and beginning of 1944 about half of the men were replacements. During the early parts of the Saidor campaign anopheline breeding was excessive and large numbers of adults could occasionally be collected. The largest catch made by the personnel of the 5th Malaria Survey Unit was 889 in one tent in an all night catch (11). This catch was exceptional.

During this period the whole division was taking .5 gm. atabrine two times weekly. That this regime was not producing protective plasma levels is apparent in Figure 2. The regime was probably not conscientiously taken. It is natural then with the swarms of anophelines, and the inadequate suppression which

allowed crescent carriers to develop (two were discovered in 400 smears) that considerable infection of the troops would take place. Yet suppression was good enough to prevent conditions approaching epidemicity as shown by the low infection rate in the anophelines, collected in many different areas. (1 stomach positive in 252 dissections.)

Two months later the II Regiment of this Division was studied at Aitape. At this time they were in the midst of a defensive campaign. In many ways conditions here were very different from those elsewhere in New Guinea. For weeks the perimeter was relatively static. Large areas of the jungle were cleared for lines of fire in front of the pill boxes. The continual creation of new breeding

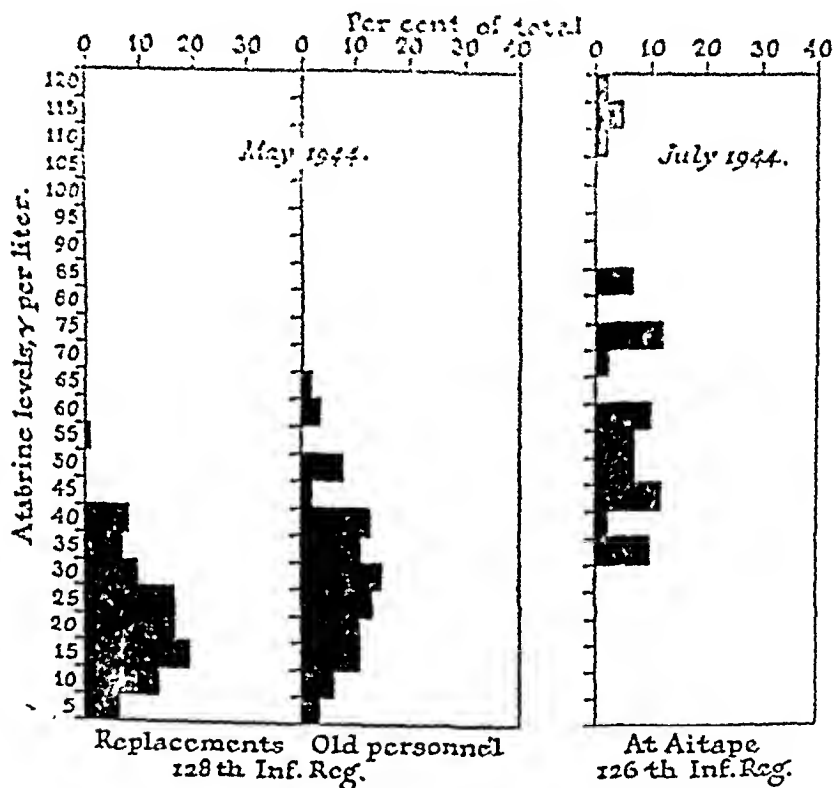


FIG. 2. ATABRINE LEVELS ON B DIVISION

places through necessary construction work in the muddy sunlit areas of the jungle out of reach of ordinary spraying made effective mosquito control very difficult. Collection of adult anophelines at this time from the jungle hammocks scattered throughout the area yielded 122 anophelines. None of these was found infected.

This regiment had had a high malaria rate during the previous month but no crescent carriers were found in the 173 men studied, although 10 per cent of the replacements were positive on careful search of thick smears (12). Atabrine levels taken from the men at random showed excellent levels (fig. 2) reflected in the drop in the malaria rate during the month.

We may reconstruct the story of this division along the following lines: They renewed their campaign with inadequate suppression, so allowed sufficient carriers to develop among the troops that when they were forced to fight in areas of high anopheline density a large proportion of the men became infected. Nevertheless suppression during both of their campaigns was good enough to smother any tendency toward an epidemic, as shown by the low rates of infection in the mosquitoes. Their story again is an example of the interrelation of mosquito control and atabrine suppression. The two are complementary and a great load is placed on either of these methods if the other is inadequate.

BASE SERVICE TROOPS

Since emphasis has been placed on the difficulty of malaria control in combat areas it is often not realized that even during "good mosquito control" in an established base, a large portion of the men can become infected. A port company which arrived in one of the bases some months after it was established and which was quartered out of the flight range of any native village became highly infected under indifferent suppression. Six months after arrival they had 17.2 per cent positive smears and had a rate of 442/1000/yr. A station hospital which had arrived in the base some months after that developed 7.1 per cent positive smears with a rate of 287/1000/yr. Due to poor suppression and little personal anti-mosquito protection these troops served as the source of their own infection and built up a high rate despite the apparent paucity of adult anophelines and the lack of contact with infected natives.

USE OF PLASMOCHIN

Plasmochin has often been used in the routine treatment of falciparum malaria with the idea of eliminating crescent carriers and thus cutting down on the spread of the infection among the troops. This would be sound reasoning if most of the gametocyte carriers found at random had previously been hospitalized for malaria, but only a small proportion of carriers found on routine surveys have been hospitalized. Of four crescent carriers we found on routine surveys and on whom it was possible to check their past history, one denied ever having been sick, one had been hospitalized for dengue and two for Fever of Unknown Origin several weeks previously. Parasites had not been detected in any of them. Thus none of these cases would have received plasmochin through routine treatment of clinical malaria in the hospital. The development of the crescent carrier state during indifferent suppression was demonstrated in another individual who on routine survey was found to have 10 rings/400 w.b.c. but was without symptoms. A repeat smear a month later showed ten crescents although he had not been sick.

AMOUNT OF FALCIPARUM A MEASURE OF TRANSMISSION DURING SUPPRESSION

It is often pointed out that with atabrine suppression exerting its dampening effect, it is impossible to know just how much transmission is going on in an area. It is suggested that greater attention be paid in the tropics to the ratio of falciparum

parum to vivax malaria as a measure of this. It is now well recognized that falciparum infections survive only a few weeks under atabrine suppression and thus cases of falciparum malaria which occur in suppressed troops must have been acquired recently. In other words, we are able to follow the rise and fall of transmission by following the percentage of falciparum malaria in troops under suppression just as well as during an epidemic in unprotected individuals. The figures in Table V show this effect. They are taken from another division during and after their campaign on Biak. This rugged coralline island has very few breeding places and for this reason transmission approached zero shortly after the landing, at the end of May. Following this their falciparum malaria waned.

TABLE V

DATE WEEK ENDING	P. VIVAX	P. FALCIPARUM	P. FALCIPARUM
			%
June 23.....	18	5	20.7
June 30.....	55	6	9.8
July 7.....	52	12	18.7
July 14.....	58	8	12.1
July 14-Aug. 11.....	226	5	2.2
Aug. 11-Aug. 25.....	109	0	0.0

SUMMARY

Atabrine suppression is not only important in suppressing individual attacks of malaria, but it has a dampening effect on the development of epidemic malaria in troops. This is demonstrable since the malaria rate in troops, the gametocyte rate in troops, and the infection rate in mosquitoes vary in direct relation to one another. Thus adequate atabrine suppression in large numbers of men prevents many infections that would otherwise take place.

Studies on two divisions during and after combat illustrate the thesis. The studies include determination of atabrine levels, thick smear surveys, mosquito dissections and malaria surveys.

The routine administration of plasmochin in acute attacks of malaria is found of little practical value in preventing transmission among troops under suppression. None of four carriers picked up on routine surveys had been treated for malaria previously. The symptomless development of the carrier state under atabrine suppression was demonstrated in one case.

Although atabrine suppression so depresses the total malaria rate that this is no indication of recent transmission, it is possible during imperfect suppression to follow transmission by following the ratio of falciparum to vivax malaria.

ACKNOWLEDGEMENTS

We are indebted to numerous people and organizations for these data. First of all Major Frank Fenner was most helpful in furnishing the figures from the Australian Army.

We are indebted to the following officers for cooperation in dissections of anophelines in their area, and for allowing us to use figures obtained while working with them.

Captain William Horsfall of the 17th Malaria Survey, Captain Harry Brown and Lt. Alan Donaldson of the 28th M.S.U. Captains Owen Graham and Malcolm Ferguson of the 5th M.S.U., and Captain Marvin Meyer and Capt H. Thomas of the 27th M.S.U.

We are indebted to the Divisional and Regimental Surgeons.

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THE TRANSMISSION OF *PLASMODIUM MALARIAE* BY *ANOPHELES MACULIPENNIS FREEBORNI*¹

MARTIN D. YOUNG² AND ROBERT W. BURGESS³

Anopheles maculipennis freeborni has been generally accepted upon epidemiological evidence as the main vector of malaria on the west coast of the United States. The experimental transmission of *Plasmodium malariae* by this mosquito is now reported.

The malaria used was the U. S. Public Health Service strain of *P. malariae* which was isolated in 1932, and since has been used continuously at this hospital for the treatment of neurosyphilitic patients (4, 8). The mosquitoes used were from a colony (F-1 strain) established from adults taken at Marysville, California (5, 6).

A. quadrimaculatus was used simultaneously as controls. The donor patient infecting the mosquitoes was a Negro in whom malaria had been induced by blood transfer. The mosquitoes were fed February 1, 1946, at which time the gametocyte count was 67 per cmm. No exflagellation of the male gametocytes was seen. The mosquitoes were kept after feeding at about 75°F.

Thirty-two days after the infective blood meal, the infected mosquitoes were applied to negro neurosyphilitic patients. The comparative data on the mosquito infections and transmission results follow:

	A. QUADRIMACULATUS	A. M. FREEBORNI
Mosquitoes dissected	44	37
Mosquitoes infected	9	5
Oocysts per gut, average	2.2	2.0
Recipient patients inoculated	1	1
Infected mosquitoes biting patients	3	3
Sporozoites per gland, average intensity*	+	+++
Patients developing infections	1	1
Prepatent period: days	29	59
Incubation period: days	28	69

* Intensity of sporozoites grouped as follows: 1-9 = +; 10-99 = ++; 100-999 = +++.

This is the third species of human malaria experimentally transmitted by *A. m. freeborni*. The other two were *P. vivax* of foreign origin (6) and *P. falciparum*, McLendon strain (3).

The experimental transmission of *P. malariae* (U. S. Public Health Service strain) by *Anopheles quadrimaculatus* has been accomplished infrequently in the past at this laboratory and also by Boyd and Stratman-Thomas (1).

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Boyd (2) reporting 5 experimental transmissions of *P. malariae* gave the range of the prepatent periods as 28 to 37 days, averaging 33 days; the incubation periods had a range of 30 to 49 days, averaging 38 days. The patient in our experiment infected by *A. quadrimaculatus* fell within this range. This is in agreement with the usual experience (7) that such relatively long developmental periods are characteristic of this species of parasite.

However, in our patient infected by *A. m. freeborni*, the periods of 59 and 69 days seem to be considerably lengthened. It is not believed that this is related to the species of the vector but rather is an indication of some immunity on the part of the patient. The latter is substantiated by the resulting infection in the patient who showed parasitemia for only 14 days, the parasites not exceeding 570 per cmm. Only 3 paroxysms were experienced during this time.

In contrast, the patient with the more normal developmental periods is still showing parasites after 58 days, with the parasites reaching 3280 per cmm. He has experienced 19 paroxysms.

SUMMARY

Anopheles maculipennis freeborni was infected with and transmitted *Plasmodium malariae*.

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THE DIAGNOSIS OF SCHISTOSOMA MANSONI INFECTIONS

NOTE ON THE USE OF A RECTAL SCRAPER¹

THOMAS H. WELLER²

Khalil and Salah el Din (1) found that the eggs of *Schistosoma mansoni* are not uniformly distributed throughout the formed stool but are most numerous in the mucus adhering to the surface. For this reason, they introduced the "rectal swab" method of diagnosis of intestinal schistosomiasis, which consisted of the microscopical examination of mucus adhering to the gloved finger following digital rectal examination. Their observations suggested that a rectal scraper might be designed which would collect a larger sample of mucus than would adhere to the gloved finger and which could be placed in a tube for transportation from the field or clinic to the laboratory. In the present note, it is desired to record a description of a rectal scraper and to report studies on the scraper method as a technique for the diagnosis of *S. mansoni* infections.

Description of the rectal scraper: Although the scraper was designed with the intent that it could be moulded from a suitable plastic material for production in quantity, the test models were machined from $5\frac{3}{4}$ inch lengths of bakelite rod having a diameter of one-half inch. The end was rounded off, and the scraper was uniformly tapered from its maximum diameter of $\frac{1}{2}$ in. at a point $\frac{3}{16}$ of an inch from the distal end to a diameter of $\frac{5}{16}$ of an inch at a point $2\frac{1}{4}$ inches from the distal end. The balance of the shaft was machined to a diameter of $\frac{5}{16}$ of an inch, except at the proximal end where it was cut down to a diameter of one-fourth inch so that the scraper would fit into a #1 one-holed rubber stopper. Following shaping, a groove $1\frac{1}{8}$ inch long (see fig. 1) was milled diagonally across the rod starting at a point $\frac{1}{8}$ inch behind the greatest diameter of the scraper and running proximally to the right lateral margin. The floor of the groove curved sharply to the surface at the distal end but was otherwise flat throughout its length. The left hand wall of the groove was smoothly beveled so that the edge was approximately $\frac{1}{32}$ of an inch below that on the right side. Originally the scraper was made with one groove; subsequently, two additional grooves were milled into the head so that identical grooves were spaced at 120° intervals around the shaft (see Figs. 1 and 2).

Method of use: Individuals were examined in a standing position with the trunk bent forward at a 90° angle. The scraper, lubricated with green soap solution, was passed through the rectal sphincter and carefully introduced for a distance of three to three and one-half inches. Then with a gentle circular movement the instrument was swung laterally around the bowel wall and at the same time rotated in a counter-clockwise direction four to six times before being withdrawn. If at any time after passage through the sphincter a definite sense

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of resistance was felt, the angle of introduction was changed and forceful insertion carefully avoided. No untoward complications resulted from the use of the scraper in the examination of a group of over one thousand men.

Tests showed that mucus and fecal material collected in the groove could be examined several hours after collection if the scrapers were fitted by their attached rubber stoppers into moist test-tubes for transportation. In the study described below, the limited number of scrapers available necessitated their immediate reuse; after each use they were scrubbed with soap and water and

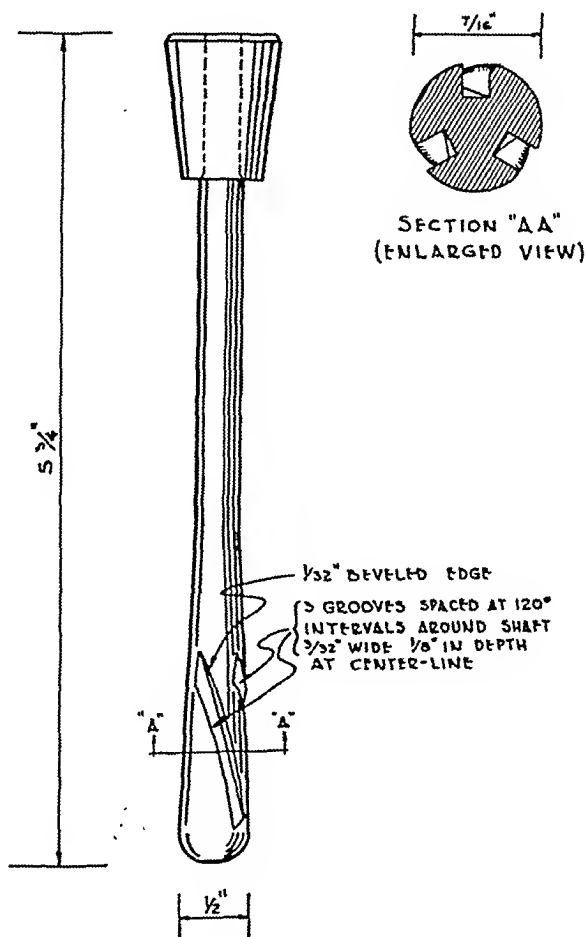


FIG. 1. DIAGRAM OF CONSTRUCTION DETAILS OF THE THREE-GROOVED SCRAPER

boiled for five minutes. Specimens could be obtained rapidly; a team of four, consisting of a doctor, two microscopists, and an assistant could collect and examine preparations from 125 men in the course of a six hour period.

Studies on the scraper method: A group of 764 Puerto Rican Selective Service registrants were examined using the single-grooved scraper; within a 24 hour period before or after the scraper examination stool samples were collected as previously described (2) and forwarded to the laboratory where they were examined by the routine acid-Triton NE-ether centrifugation technique (3).

The material collected by the scraper was removed from the groove using a wood applicator stick and emulsified in a drop of normal saline on a slide; a 22 x 22 mm. coverslip was applied and the whole area systematically surveyed for schistosome eggs using a magnification of 100X. Of the 157 men found to have schistosomiasis, the scraper method detected 94 (60 per cent), while the fecal examination technique detected 119 (76 per cent); 56 men were positive by both methods.

Studies were then initiated to test the effect of acid-ether concentration of material collected using the three-grooved scraper; the results of preliminary



FIG. 2. PHOTOGRAPH OF THE THREE-GROOVED SCRAPER

tests indicated that the combined technique was impractical because of the loss of eggs occurring during the manipulation of the relatively small amount of material. However, the use of the improved scraper on a group of over 200 men demonstrated that with the three-grooved instrument there was an increased likelihood of obtaining a mucus sample with a minimum of fecal material. Although circumstances prevented further investigation, it appears probable that the direct examination of a larger volume of material as collected by the three-grooved scraper would significantly increase the efficiency of the scraper method.

The possibility that the scraper might prove of value as a convenient means of obtaining material for the study of intestinal protozoa was raised by the in-

cidental observation that many of the saline preparations contained motile flagellates and in one of the few studied under higher magnification numbers of active *E. histolytica* were found.

SUMMARY AND CONCLUSIONS

A rectal scraper is described which was found to be a satisfactory instrument for the collection of specimens of mucus and fecal material from the rectal wall and for their transportation from the field to the laboratory. The direct examination of material collected with a single-grooved scraper was found to be less efficient than the routine acid-Triton NE-ether method of stool concentration for the detection of *Schistosoma mansoni* eggs, although the scraper method detected a significant number of positives missed by the concentration technique. Further investigation of the scraper method is indicated. In its present state, the scraper technique may prove to be a useful complement to stool concentration methods in the diagnosis of *S. mansoni* infections and should be of value in situations where the collection of fecal specimens is impractical or facilities for stool concentration are lacking.

ACKNOWLEDGEMENTS

The author is indebted to Capt. Ernest Dodson, Jr., SC, and to Sgt. S. E. Geyer of the Signal Corps Repair Shops, Antilles General Depot, for assistance in manufacturing the scrapers and to Major Carlos A. Pons, MC and Capt. Felix Reyes, MC for aid in collecting the specimens.

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MATERIAL

Seven soldiers with proven active bisexual *S. japonicum* infections were studied in an Army General Hospital in the Zone of the Interior. There was no selection of cases. They had had no treatment prior to their study here. Five had first been exposed to known infectious water 44 months previously in the Davao area of Mindanao; two had apparently been infected about 16 months previously on Leyte. Six were white and one colored. Their ages varied from 21 to 38. All were males. Other coexisting diseases were present at the time of study in three patients, namely, ascariasis, trichocephaliasis, scrotal abscess, and anxiety reaction. Following the establishment of the diagnosis and the completion of certain studies, treatment with tartar emetic was instituted. The routine treatment course included 0.16 Gm of the drug intravenously three times weekly to a total of 2.08 Gm, but toxic reaction occasionally necessitated temporary interruption of the schedule.

As controls, seven soldiers who had been stationed for significant lengths of time in known endemic schistosomiasis regions, who were being investigated regarding infection on epidemiologic grounds, and who were eventually proven by the criteria stated below to be free of infection, were chosen at random. All were white males aged 21 to 39. Four had other diseases: rectal polyp, anal fissure, eczematoid dermatitis, duodenal ulcer, and trichocephaliasis.

METHOD

The criteria for presence of active infection was the demonstration of eggs containing active miracidia, either in the stool or in material obtained by rectal crypt aspiration (Hollands and Palmer, 4). The criteria for absence of schistosomiasis was a minimum of six negative rectal crypt aspirations, the material being examined by concentration by centrifugalization, plus a minimum of 36 negative stools, each examined by direct smear, modified zinc sulfate flotation (Otto, Hewitt, and Strahan, 6), and concentration by centrifugalization technics.

A minimum of two gastroscopic examinations was made on each patient, with an average of 2.9 per infected case and 2.4 per control. Each of the 37 examinations reported was complete and satisfactory, and included visualization of the pylorus. The gastroscopies were performed with the Wolf-Schindler instrument.

Fasting gastric contents, removed by gravity drainage before gastroscopy from five patients with known infections, were examined for schistosome eggs by direct smear and concentration by centrifugalization.

X-ray studies consisted in each case of pre-treatment upper gastro-intestinal fluoroscopic and roentgenographic series.

RESULTS

The clinical gastrointestinal findings in the seven cases of proven infection were nil; no patient had upper or lower gastrointestinal complaints or positive physical findings at the time schistosomiasis was diagnosed. Among the control patients there were no gastrointestinal symptoms or signs except those of anal fissure in

one patient and of typical duodenal ulcer in another (control patient No. 7, Table II).

Gastroscopy revealed pathology which was of more than mere traumatic nature in two of the infected patients and in one of the controls (Tables I and II).

TABLE I

Findings on serial gastroscopic examination. Seven patients with active schistosomiasis

PATIENT	DAYS SINCE PREVIOUS EXAM	TOTAL TARTAR EMETIC	POSITIVE FINDINGS
1		gm.	
	5	0 .04	Excess mucus distributed over all areas Normal
2		.32	Normal
	8	.32	Group of 3 bright petechiae on greater curvature pars media, each $\frac{1}{2}$ as broad as ruga
	11	.32	As before, but lesions now 3 times as large
	18	1.44	No petechiae. Superficial erosion 3 times rugal width high on posterior wall. Small area prominent veins middle anterior wall without usual changes of atrophy; area is of normal color and venous channels are not blue
	12	2.08	2 pigmented spots one rugal width on lesser curvature
3		2.0	Normal
	4	2.08	Normal
	45	2.08	Normal
4		0	Normal. Tobacco grains present
	5	.04	Normal
5		.04	Normal
	4	0.16	Normal
6		0	Normal
	4	.04	Normal
	50	2.08	Normal
7		.48	Few scattered areas of superficial erosion about 2 rugal widths in diameter, with considerable bleeding
	10	1.12	Normal
	60	2.08	Normal

Infected patient No. 2 had changes which were consistent with the rather common picture of superficial erosive gastritis, plus venous prominence without mucosal atrophy, the latter being found five weeks after the subsidence of a mild attack of acute, probably infectious, hepatitis which had interrupted antimony ther-

apy. Infected patient No. 7 also had evidence of mild superficial erosive gastritis, as did control patient No. 2. The details are given in the tables. No lesion which could not be explained on the basis of well-known simple gastric pathology was found.

Examinations of five samples of fasting gastric contents were negative for schistosome eggs.

X-ray studies showed no structural or functional gastric abnormality in any of the infected or control patients.

TABLE II

Findings on serial gastroscopic examination. Seven control patients without schistosomiasis

PATIENT	DAYS SINCE PREVIOUS EXAM	POSITIVE FINDINGS
1	6	Normal Normal
2	18 15 45 45	Normal Small scattered areas hyperemia with tiny bleeding erosions on anterior wall pars media No change in previously observed lesions No erosion or bleeding. Rugae of prepyloric region along greater curvature are hyperemic Scattered rugae along greater curvature are hyperemic
3	7	Normal Normal
4	9	Free blood of undetermined origin, probably traumatic Normal
5	6	Normal Normal
6	24	Normal Normal
7	6	Tiny hemorrhage on greater curvature in proximal an- trum, apparently traumatic Normal

COMMENT

The positive gastroscopic findings which are reported cannot be construed as evidence of schistosomal pathology because they are found commonly in several classes of general medical patients and were seen in one of the controls in this series. The question as to whether the absence of demonstrable gross gastric pathology necessarily indicates absence of involvement of the gastric veins has not been answered. The possibility that there may be no close correlation between local involvement and local gross mucosal changes in the stomach must be

considered because Hollands and Palmer (4) have reported instances of complete dissociation in the case of the rectum.

CONCLUSION

As a result of controlled X-ray and serial gastroscopic studies on seven soldiers with proven active schistosomiasis japonica, it was found that the infection did not produce grossly demonstrable lesions in the stomach. It seems likely from available evidence that in relatively early and presumably light infections, such as seen in American military cases, the mature worms generally do not inhabit levels of the gastrointestinal venous system as high as the stomach.

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INTRADERMAL AND COMPLEMENT FIXATION REACTIONS ELICITED BY VARIOUS ANTIGENS IN PERSONS INFECTED WITH *ONCHOCERCA VOLVULUS*¹

JOHN BOZICEVICH,* ANTHONY DONOVAN, LUIS MAZZOTTI,
FRANCISCO DIAZ A., AND ENRIQUE PADILLA

Various authors have attempted to employ the intracutaneous and complement fixation tests for diagnosing onchocercosis but conflicting results have been obtained. Most of these authors arbitrarily selected certain antigen dilutions for these tests and no attempt was made to perform quantitative titrations with various dilutions of the antigens in order to screen out non-specific reactions due to toxicity of the material or the influence of parasitic infections other than *Onchocerca volvulus*. In addition to an investigation of this factor, it seemed desirable to obtain a more accurate measure of the specificity of *Onchocerca volvulus* antigen and to determine the efficacy and specificity of antigens prepared from such readily available filariids as *Dirofilaria immitis*, *Setaria equina*, and *Litomosoides carinii*.

This study is a continuation of the investigation begun by Wright and Murdock (1) in the research and control of onchocercosis in cooperation with the governments of Mexico and Guatemala. The above authors have summarized the literature up to the time of publication of their article. Consequently, no review of the literature will be given at this time with the exception of two references not published until after the appearance of the paper by Wright and Murdock (1).

Mazzotti and Ozorio (2) prepared an antigen from *O. volvulus* by digesting recently extirpated cysts containing adult worms and extracting the desiccated worm powder according to the method of Bozicevich (3). Two dilutions of the antigens were made and tested intracutaneously on 116 individuals residing in endemic zones, while 117 control individuals were tested in nonendemic areas. When antigen I (1:10,000 without merthiolate) was used 28, or 70 per cent, of 40 individuals without nodules gave positive reactions while 19, or 62 per cent, of 31 patients with nodules reacted positively. When antigen II (1:8,000 with merthiolate) was employed positive reactions were obtained in all of 32 individuals without nodules and in all of 13 individuals with nodules. However, of 71 control individuals tested with antigen II, 13 gave false positive reactions; on the other hand, antigen I gave only 1 false positive in 36 patients tested. The above authors concluded that their antigens were not useful for the diagnosis of onchocercosis because of the number of non-specific reactions elicited. They believed that further studies should be made on this problem but that the disease ordinarily presents clinical evidences of the infection that can be confirmed by recovery of microfilariae on biopsy or xenodiagnosis.

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² Assigned from the Zoology Laboratory, National Institute of Health, Bethesda, Md.

Culbertson, Rose, and Demarest (4) prepared an antigen from *Litomosoides carinii*, a nematode found in the thoracic cavity of the cotton rat, *Sigmodon hispidus*. They used a 1 per cent emulsion of the dried pulverized worms in physiologic saline preserved with 0.5 per cent phenol. Their control solution consisted of physiologic saline with 0.5 per cent phenol. However, 2 individuals only were tested intracutaneously; one had possessed 2 onchocerca nodules which had been removed from the scalp, while the other had a small circumscribed nodule at the base of the scalp. While it is difficult to place any significance on tests conducted on only 2 individuals, the results of their control series of 40 persons not exposed to onchocercosis are of more importance. Thirty-eight of the 40 gave no response, but 2 individuals gave distinct wheals with pseudopodia. The above authors concluded that since the antigen seldom elicited positive intradermal reactions in normal individuals, it should be useful in the diagnosis of filarial infections.

PREPARATION OF THE ANTIGENS

Three of the 4 antigens used in our study were prepared according to the method given by Bozicevich and Hutter (5) but the technique for the preparation of the *O. volvulus* antigen necessitated some modification. After extirpation from infected patients, the nodules were transported in sterile containers within thermos jars packed with ice to the laboratory of the *Centro Medico y de Investigaciones sobre le Onchocercosis*, at Huixtla, Chiapas, Mexico, where an effective method for the separation of the filariids from the nodules was used. The nodules were placed in artificial gastric juice and the digestion was observed for various periods of time. As soon as the fine sediment of the digested material was evident, the artificial gastric juice was changed. This was performed as often as was necessary for a period of 36 to 48 hours. In the meantime, as the worms were liberated from the cysts, they were removed in order to avoid unnecessary digestion of the nematode tissue. After separation, the worms were frozen in the ice cube freezing compartment of a refrigerator. When a sufficient amount of the material had been accumulated, the cubes were thawed and the worms placed in a bottle containing a small amount of distilled water. The bottle was packed in a large iced thermos jar and shipped by air express to Bethesda, Md. with re-icing at one point en route. The material was then treated in the same manner as that from the other filariids with the exception that neutralization was necessary because of the acidity acquired from the artificial gastric juice.

Considerable care must be exercised in the preparation of *O. volvulus* antigen since material from worms remaining too long in the digestive fluid will produce an antigen of lower potency. All *O. volvulus* antigens employed in the present experiments were standardized against anti-onchocerca rabbit serum.

There is some possibility that host protein might be carried over in the body cavity of the worms. In order to eliminate this factor, a control protein solution was prepared for each antigen employed in the intradermal tests. Dog serum, was used as a control for *D. immitis* antigen, horse serum for *S. equina* antigen and rat serum for *L. carinii* antigen.

Because of the fact that phenol was used in the preservation of the antigens and host protein control solutions, a saline control containing 0.3 per cent phenol was also employed.

TECHNIQUE OF TESTS

Intradermal tests: All antigens and host protein control solutions were employed in a 1:8,000 dilution with the exception of a second *O. volvulus* antigen which was diluted to 1:16,000.

The volar region of the forearm was rubbed with 70 per cent alcohol and the test antigens and control solutions were injected. Each one of the solutions was injected at a site separated from the other sites by a distance of about 5 cm. The control host protein solutions were always injected in a site opposite the specific test antigen. The *O. volvulus* antigens were injected opposite each other and the control saline solution beneath these two antigens.

All solutions were injected intradermally in amounts of 0.01 to 0.03 cc., i.e., in a quantity sufficient to raise a very small wheal approximately 1 to 2 mm. in diameter. The reactions were read 8 to 12 minutes after injection. Standards for evaluating the reactions are discussed later in this paper but in no case was a reaction considered positive unless the diameter of the antigen wheal exceeded that of the control wheals by 3 mm. or more. The wheals were usually well defined, firm, raised areas with or without pseudopodia; if the skin was drawn taut, a blanched area could usually be seen. In some instances, erythema was evident but, because of the dark character of the skin of the patients, this feature was not considered in evaluating the reaction.

Complement fixation tests: In conducting the complement fixation studies, attempts were made to employ all the above-mentioned antigens but it was soon discovered that the *Setaria equina* antigen contained markedly anticomplementary properties even in a dilution of 1:2,000 and could therefore not be used. Our supply of *L. carinii* antigen was not sufficient for use in complement fixation tests.

Quantitative complement fixation tests were performed on a number of serums obtained at Yepocapa, Guatemala, with both the *D. immitis* and *O. volvulus* antigens. The antigens were diluted serially from 1:200 to 1:4,000 and the serums from 1:2 to 1:32. Varying dilutions of both the serum and antigens in 0.2 cc. amounts were used in each test. Two units of guinea pig complement contained in 0.2 cc. were added to each tube after the complement was titrated in the presence of the most concentrated antigen to be employed in the test. The tubes were then placed in the refrigerator at a temperature of 6 to 7° C. and allowed to remain overnight. The next morning the tubes were removed and placed in a water bath at 37° C. for 1 hour. After removal from the water bath, the hemolytic system consisting of 0.2 cc. of a 2.5 per cent suspension of sheep cells and 2 units of amboceptor contained in 0.2 cc. was added to each tube. The tubes were replaced in the 37° C. water bath for an additional hour when they were removed and read. Controls tests were performed on negative serums and on the antigen for anticomplementary and hemolytic effects.

Since we employed the overnight fixation method, it was desirable to establish

the exact amount of complement bound by each serum tested. To this end, control serum tests were performed so that to each serum dilution employed in the test complement was added to give 1, 1.5, and 2 units. The total volume of the control test was the same as in the test proper. In these control tests, the volume was made up to that in the test proper by the addition of equivalent amounts of saline to replace volume normally occupied by the antigen. This procedure was repeated with all antigen dilutions employed. As a final control, we also tested the complement binding power of the saline used in preparing the dilutions.

A reading of 3 plus fixation or better was regarded as a criterion for a positive result.

RESULTS OF INTRADERMAL AND COMPLEMENT FIXATION STUDIES

Tests at Yepocapa, Guatemala: In Guatemala the intradermal tests were conducted at the town of Yepocapa. A routine procedure was inaugurated which involved taking a blood sample for the complement fixation test for onchocercosis and obtaining a skin biopsy from the ear of the patient prior to conducting the intradermal test. The piece of skin was placed in a drop of saline on a slide, teased with dissecting needles, and examined under the microscope. If the preparation did not reveal the presence of microfilariae at this time, the slide was placed in a humidor and examined every 15 minutes until a period of several hours had elapsed. In several instances it required a period of 5 hours before microfilariae could be seen in the skin section. If the biopsy contained numerous larvae, these became evident immediately under the microscope, whereas if the sample contained only a few larvae, a longer period of search was required.

The intradermal tests followed the biopsy and immediately after these tests the nodules were extirpated and dissected to determine the presence of *Onchocerca*. Thus, it was possible to correlate the results of the skin biopsy with the complement fixation and intradermal results on individuals who definitely had onchocercosis as disclosed by the presence of adult worms in the extirpated nodules.

In the Yepocapa investigation, individuals were divided into two series. The first series included 158 adults and school children who had nodules and from whom a group of 81 was taken for the comparative studies. The second series included 107 school children with no visible or palpable nodules. Fecal examinations were made on 86 of the 107 school children.

In the group of 81 individuals on whom biopsies were taken, only 33, or 40.7 per cent, revealed the presence of microfilariae. Only one biopsy was taken on each person as time did not permit additional ones and this fact should be kept in mind when appraising the results. Most of these individuals had only 1 nodule; only 24 had 2 or more nodules. Notwithstanding, some of these individuals showed numerous microfilariae on skin biopsy. The results of the comparative studies are shown in table 1.

If the group of 81 individuals is replaced in the original series from which it was taken, then the number and per cent of individuals eliciting a positive intradermal test is shown in table 2-A.

The complement fixation test with *O. volvulus* antigen in dilutions of 1:200, 1:100, and 1:800 was conducted on 60 of the 81 individuals. In every instance a strong positive complement fixation was recorded in serum dilutions ranging from 1:4 to 1:32. *D. immitis* was employed as the antigen in the complement fixation tests on 53 serum samples, of which 49, or 92.4 per cent, gave positive reactions. Positive intradermal tests with *D. immitis* antigen and the other antigens were obtained on the 4 individuals showing negative complement fixation tests with *D. immitis* antigen.

In comparing the sensitivity and specificity of the *D. immitis* and *O. volvulus* antigens as employed in the complement fixation test, it was found that the *O. volvulus* antigen gave positive results in all of the 53 cases tested with the *D. immitis* antigen while the latter yielded only 49 positives. Furthermore, when the *O. volvulus* was employed the serum dilutions were greater and the antigen could be diluted further than in the case of the *D. immitis* antigen.

Two individuals not included in the above series of 158 had no palpable nodules

TABLE 1

Number and per cent* of infected individuals showing positive skin biopsies and intradermal tests to various antigens (Yepocapa group with nodules)

NO. OF CASES	NO. OF POSITIVE BIOPSIES	D. IMMITIS 1:8,000			S. EQUINA 1:8,000			L. CARINI 1:8,000			O. VOLVULUS 1:8,000			O. VOLVULUS 1:16,000		
		3 mm.†	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.
81	33	67	56	46	58	45	31	46	23	17	78	65	49	71	53	30
Per cent positive....	40.7	82.7	69.1	56.7	71.6	55.5	38.2	56.7	28.3	20.9	96.2	80.2	60.4	87.6	65.4	37.0

* In view of the small number of persons involved, percentage figures are used for convenience only.

† Figures indicate the excess diameter in mm. of the antigen wheal over that of control wheals.

and gave negative intradermal tests to all the antigens but showed numerous microfilariae and gave positive complement fixation tests with both the *D. immitis* and *O. volvulus* antigens.

The results of the intradermal tests on the 107 Yepocapa school children who had no demonstrable nodules are shown in table 2-B. It must be remembered that the above-mentioned children resided in a heavy endemic zone of onchocercosis although skin biopsies taken a month previously disclosed no microfilariae in any instance.

Since it has been previously suggested that infection with other helminths might have some influence on the results of the intradermal tests, stool specimens were obtained from eighty-six of the series of 107 school children and examined for parasite ova. A 100 per cent infection with both *Ascaris lumbricoides* and *Trichuris trichiura* was present while 49 of the 86 harbored hookworms. Consequently, 49 harbored a triple infection and 86 had a double infection.

Tests at Huixtla, Mexico: The high incidence of positive intradermal reactions

obtained on the Yepocapa school children who harbored intestinal parasites necessitated additional investigation with such subjects. Accordingly, 57 school children at Huixtla, Chiapas, Mexico were tested. While no focus of *Onchocerca* infection exists at Huixtla, it was later found that some of the children were

TABLE 2

Number and per cent* of infected and control individuals exhibiting positive intradermal reactions to various antigens

NO. OF CASES	D. IMMITIS 1:8,000			S. EQUINA 1:8,000			L. CARINI 1:8,000			O. VOLVULUS 1:8,000			O. VOLVULUS 1:16,000		
	3 mm.†	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.	3 mm.	4 mm.	5 mm.
A. Individuals with nodules and those who had nodules removed (Yepocapa series)															
158	124	104	85	110	81	54	94	59	45	150	127	90	134	93	47
Per cent	78.4	65.8	53.7	69.6	51.2	34.1	59.4	37.3	28.4	94.9	80.3	56.9	84.8	58.8	29.7
B. Yepocapa school children															
107	53	40	30	53	41	23	68	52	32	72	54	27	62	33	13
Per cent	49.5	37.3	28.0	49.5	38.3	21.4	63.5	48.5	29.9	67.2	50.4	25.2	57.9	30.8	12.1
C. Huixtla school children															
57	22	14	8	15	7	4	22	9	4	25	18	8	17	10	5
Per cent	38.5	24.5	14.0	26.3	12.2	7.0	38.5	15.7	7.0	43.8	31.5	14.0	29.8	17.5	8.7
D. Mexico City control series															
209	30	14	7	33	15	5	25	7	2	15	7	5	11	5	1
Per cent	14.3	6.6	3.3	15.7	7.1	2.3	11.9	3.3	0.9	7.1	3.3	2.3	5.2	2.3	0.4
E. Tamazunchale school children															
127	31	7	1	42	21	7	41	9	1	40	12	8	21	7	1
Per cent	24.4	5.5	0.7	33.0	16.5	5.5	32.2	7.3	0.7	31.5	9.4	6.2	16.5	5.5	0.7
F. Morganton, N. C. control series															
50	10	6	4	11	6	4	6	3	1	2	1	0	1	0	0
Per cent	20.0	12.0	8.0	22.0	12.0	8.0	12.0	6.0	2.0	4.0	2.0	0.0	2.0	0.0	0.0

* In groups with less than 100 individuals, percentage figures are used for convenience only.

† Figures indicate the excess diameter in mm. of the antigen wheal over that of control wheals.

from families who had migrated from endemic areas; consequently the information obtained from this so-called control group was invalidated by previous exposure to onchocercosis of some of the individuals. Forty-eight of the 57 children harbored parasites other than *O. volvulus*; 12 had hookworms, 29 had

Ascaris, 30 had *Trichuris*, and 9 had *Strongyloides stercoralis*. From these figures it can be seen that the majority of them had multiple infections. However, the number of individuals was too small to attempt analysis. Nevertheless, for reasons mentioned later the number of individuals giving positive intradermal tests is shown in table 2-C.

Tests at Mexico, D. F.: Because we did not possess a true control series of individuals up to this point, intradermal tests were conducted on control individuals in Mexico City at *Hospital Juarez, Escuela Francisco y Madero*, and the charity home, *Internado Nacional Infantil*. None of the individuals tested had ever been in endemic zones of onchocercosis to the best of his knowledge, but in testing this group two children gave strong intradermal reactions. In questioning them, it was found they had migrated from the State of Guerrero and therefore the sample of population tested did not consist altogether of natives of Mexico City. A total of 209 individuals was examined in this series. Since the number of individuals examined at any one place was small, all three groups are combined as a single control unit. In addition to the fecal examinations, anal swabs were made on the group at the *Internado Nacional Infantil*. Doctor Mazzotti had made periodic examinations of this group and found that practically all of them at one time or another had harbored *Enterobius vermicularis*. The results of the examination for parasites were as follows: Fifty-one harbored *Ascaris*, 31 had *Trichuris*, 18 had *Enterobius*, 6 had *Hymenolepis nana*, and 4 had hookworms. A total of 92 individuals had single or multiple parasitic infections. Table 2-D shows the results of the intradermal tests with various antigens on the Mexico City group.

Tests at Tamazunchale, Mexico: Because of the relatively small number of parasitized control individuals, we decided to test additional parasitized children who had never been in endemic zones of onchocercosis. This opportunity was afforded us at Tamazunchale, Mexico, where a survey had just been completed by the Pan American Highway survey group under the direction of Doctor H. J. Bush. This series consisted of 127 children, of whom 100 had *Ascaris*, 108 had *Trichuris*, 76 had hookworms, 12 had *H. nana*, and 3 harbored *Taenia* spp. The results are shown in table 2-E.

At the time of conducting the intradermal tests we were not prepared to draw blood samples from the above-mentioned patients but these were taken approximately 4 months later. It has been the experience of some of us that repeated intradermal tests with relatively weak dilutions of antigen do not sensitize individuals; since these children only received one test, this factor can be eliminated. Twenty-two of 50 available serums gave positive complement fixation tests with *D. immitis* antigen. Due to the lack of *Onchocerca* antigen only 8 could be tested and these gave positive complement fixation tests with both antigens. Due to circumstances beyond our control, the blood samples were delayed in transit and the majority became markedly hemolyzed. This factor may have influenced the results of the tests.

Tests at Morganton, N. C.: Because of the anomalous results obtained with the intradermal and complement fixation tests on the Tamazunchale school

children harboring helminth parasites other than *O. volvulus*, it was apparent that more work was needed to evaluate the possibility of false positive reactions in such cases. Through the courtesy of Doctor J. R. Saunders, Superintendent, State Hospital at Morganton, N. C., we were able to conduct intradermal and complement fixation tests on 50 patients, of whom 31 were infected with *Trichuris*, 20 with *Enterobius*, 7 with hookworms, 1 with *Hymenolepis nana*, and 1 with *Strongyloides*. So far as could be ascertained, none of these individuals had ever been exposed to *Onchocerca volvulus* or any other filariid infection. The intradermal antigens were the same as those used on the groups in Guatemala and Mexico. Results of the intradermal tests are summarized in table 2-F.

Blood samples were drawn from 49 of the above-mentioned individuals; 3 gave positive complement fixation reactions, 1 was doubtfully positive, 4 samples were anticomplementary, and insufficient amount of serum was furnished in 2 cases, while 39 proved to be negative. The tests were done with *D. immitis* antigen only.

DISCUSSION

It is clearly shown in table 1 that antigen prepared from *O. volvulus* is more sensitive than are antigens prepared from the other filariids. The second best antigen appears to be the one prepared from *D. immitis*. Non-specific reactions occurred more frequently with *D. immitis* antigen than with the others employed in the intradermal test; however, it appears that when the false positives and false negatives of the intradermal test are grouped together and evaluated with respect to the false negatives of the single biopsy method, approximately 18 per cent false reactions were obtained with the former while 60 per cent false negatives were obtained with the latter. The above false reactions with *D. immitis* occurred when a wheal 3 mm. larger than the control was considered as the criterion of a positive reaction. In some instances, the *L. carinii* antigen gave good results but it displayed a marked variation in reactions in both the infected and control individuals. It is believed that this variation may be partly explained by the method of preparation of the antigen and suggests that the technique should be altered. Since all the antigens were extracted in the same initial dilution, the residue remaining after extraction should have been somewhat the same. It was found, however, that less residue remained after the preparation of *L. carinii* antigen and that the supernatant fluid was more opalescent. In other words, the material was in a finer state of colloidal suspension and this suspension was not entirely removed by the procedures employed. Therefore, if larger colloidal particles are injected into a control subject the chance of obtaining a false positive reaction is enhanced.

The effect of dilution can be seen from the difference in the number of individuals giving positive intradermal reactions with the 2 dilutions of the *O. volvulus* antigens. This effect is further noted in the decrease in the number of individuals in the control series giving false positive reactions with the 1:16,000 dilution. It would appear that a dilution between the 1:8,000 and 1:16,000, say 1:10,000 or 1:12,000, would give better results. Another alternative which

might be used to increase the specificity of the intradermal tests with the 1:8,000 dilution would be to employ the 4 mm. wheal diameter as a criterion of a positive reaction. Using this measurement, fewer false positive reactions occurred than with the 1:16,000 dilution and 3 mm. wheal diameter criterion.

If we consider a positive reaction as one in which the diameter of the antigen wheal exceeded that of the control wheal by 3 mm. or more, the 209 control individuals in Mexico City gave a considerable number of false positive reactions with the *D. immitis* and *S. equina* antigens and a smaller number with the *O. volvulus* antigens. In fact, the number of such reactions with the two former antigens represented twice the number obtained with these same antigens in a control group of patients suffering from various allergic conditions. These patients were from the Allergy Clinic of Walter Reed Hospital, Washington, D. C. and were tested through the courtesy of Colonel L. E. Leider. Consequently, it is believed that the possible presence of allergic conditions in the Mexico City group was less responsible for the false positive reactions than was the infection with parasites other than *O. volvulus*. Some of these control individuals not harboring intestinal worms at the time of the test also gave false positive reactions. It is possible that such reactions were due to a previous infection with intestinal worms, since it is known that skin reactions to a specific worm antigen may be obtained for a considerable period of time after the infection has been eliminated.

Thus the evidence accumulated in this work substantiates the belief that a certain percentage of false positive reactions may be elicited when intradermal tests with filariid antigens are conducted on individuals harboring helminth parasites other than *O. volvulus*. The examination of the school children at Tamazunchale, Mexico, adds confirmation to this view. This is especially evident if the criterion of the 3 mm. difference in wheals is used to evaluate the tests.

The results of the complement fixation tests on the Tamazunchale group support the findings on the intradermal tests. The 22 positive reactions in the 50 serum samples cannot be explained on the basis of present evidence. The large number of positive tests cannot be attributed only to the use of *D. immitis* antigen because in the United States false positive reactions on the complement fixation tests have been encountered in only 5 to 7 per cent of individuals in which this antigen has been employed. This strongly suggests that comparative studies should be made on the Tamazunchale group with *O. volvulus* antigen; however, such studies could not be carried out because of an insufficient amount of material. There was some difference in the titer of reactions obtained with the complement fixation in the Tamazunchale series and that noted in the Yepocapa series of individuals with *O. volvulus* nodules. In the former, positive reactions resulted with the use of a 1:4 dilution of serum and a 1:200 dilution of the antigen. On the other hand, in the Yepocapa series, the *D. immitis* antigen gave positive reactions with serum dilutions as high as 1:32.

The Morganton series of individuals harboring helminth parasites other than *O. volvulus* gave a high percentage of false positive intradermal reactions with the

D. immitis and *S. equina* antigens when judged both by the 3 mm. and 4 mm. wheal criteria. The tests with the *L. carinii* antigen gave fewer positive reactions and a further decrease in the percentage of positives was noted with the two dilutions of the *O. volvulus* antigen. The complement fixation tests gave only 6 per cent false positive reactions, a much lower figure than obtained in the Tamazunchale series.

In considering the cause of the anomalous results obtained in the Tamazunchale series, four possibilities are suggested. The first possibility is that onchocercosis is endemic in the area and that the large number of positive reactions was due to the presence of this infection. However, the Mexican Government has maintained and supported a Federal Health Center at Tamazunchale for the past 10 years and the presence of onchocercosis in the area has never been recorded. Second, it might be possible that *D. immitis* occurs in dogs in the community and that some of the individuals tested might have become sensitized through the bites of the intermediate mosquito hosts. The third point concerns the influence of intensity of infection with intestinal helminths on intradermal and complement fixation reactions. The majority of the control group disclosed only medium or slight infections of intestinal parasites, and in this small group no correlation was noted between their intradermal reactions and the degree of intestinal infections. Little is known concerning the matter and more studies are indicated. Finally, it is also possible that nutritional deficiencies influenced the results in this group, since youngsters in a private school at Tamazunchale gave fewer positive intradermal reactions than did those who had a lower economic status and attended public schools.

If reasons for the results in the Tamazunchale group become evident and a more specific complement fixation test can be made available, it would appear that this would be of value in supplementing skin biopsies in following the course of experimental therapy in onchocercosis. At the present time the skin biopsy is the only method available for this purpose. Death of adults and microfilariae would be followed by a gradual decrease in circulating antibodies and a reliable complement fixation test would be of value in following disappearance of antibodies as a measure of chemotherapeutic efficacy.

In concluding this discussion, it should be noted that Mazzotti and Osorio (2) failed to obtain as high a percentage of positive intradermal reactions as encountered in the present studies. The former authors prepared their antigen according to the method recommended by Bozicevich for the preparation of trichina antigen. The antigens used in the present work were made up in a somewhat different manner since it was found that alternate freezing and thawing along with inactivation at 56°C. for 4 hours produced an antigen which was less irritating and less anticomplementary.

SUMMARY AND CONCLUSIONS

In order to evaluate the two methods, a total of 658 intradermal and 161 complement fixation tests was conducted on onchocercosis patients in endemic areas and on control individuals in other areas.

A single skin biopsy on 81 individuals with *Onchocerca* nodules disclosed microfilariae in 40 per cent of the cases, whereas *O. volvulus* antigen in a dilution of 1:8,000 gave a positive reaction in 78, or 96.2 per cent, of the individuals when the reading of the reaction was based on an antigen wheal greater by 3 mm. or more than the control wheal. In a dilution of 1:8,000, antigens prepared from *Dirofilaria immitis*, *Setaria equina*, and *Lilomosoides carinii* gave a lower percentage of positives; the efficacy of these antigens was exceeded by a 1:16,000 dilution of *O. volvulus* material.

The *O. volvulus* antigen was more specific than the other antigens. In a series of 50 patients (Morganton, N. C.) who had never been exposed to *O. volvulus* infection but who harbored other helminth parasites, false positive reactions were obtained with a 1:8,000 dilution in the following number of cases: *D. immitis* 10, *S. equina* 11, *L. carinii* 6, and *O. volvulus* 2. In a Mexico City group of 209 individuals not exposed to *O. volvulus* but of whom 92 were infected with intestinal helminths, twice as many false positive reactions were obtained with *D. immitis* and *S. equina* antigens than with the *O. volvulus* material.

Complement fixation reactions with *O. volvulus* antigen proved positive in all of 60 individuals with *Onchocerca* nodules; *D. immitis* antigen gave positive reactions in 49 of 53 of these cases. In a control group of 43 persons infected with helminth parasites other than *O. volvulus*, *D. immitis* material gave positive complement fixation tests in 4 cases, a doubtfully positive reaction in 1 case, and a negative reaction in 39 cases.

Intradermal and complement fixation tests on a series of school children at Tamazunchale, Mexico, not supposed to be within the endemic area of onchocercosis, gave a higher percentage of positive reactions than obtained in individuals in any other control group. At the present time no satisfactory explanation can be given for the significant difference in the number of positives obtained in the control individuals at Tamazunchale and Mexico City.

From the results in all series, it appears that the *O. volvulus* antigen is more specific and sensitive than the other three antigens employed in the intradermal tests. The second best antigen appears to be that from *D. immitis*. The *O. volvulus* antigen is also superior to the *D. immitis* antigen when employed in the complement fixation test. Because of the lack of material, judgment on the comparative value of *L. carinii* antigen in complement fixation tests must await further work. Antigen prepared from *S. equina* invariably gave anticomplementary reactions.

Quantitative complement fixation tests are suggested as a method of measuring the efficacy of drugs in studies on the chemotherapy of onchocercosis.

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INTESTINAL PARASITIC INFECTIONS IN NAVAL PERSONNEL^{1, 2}

EDWARD K. MARKELL,³ PAUL E. MULLINGER⁴ AND DONALD J. SCHNEIDER⁵

When the School of Tropical Medicine was established at the Naval Hospital, Treasure Island, in July, 1944, it became necessary to have a source of parasitic material for instructional purposes. Accordingly, the school undertook the examinations for intestinal and blood parasites previously done by the hospital's clinical laboratory. This not only provided material for class use, but also gave the authors a chance to secure data on the incidence of parasitic infections in a rather representative group of naval personnel, drawn from the various activities of the Twelfth Naval District.

The total number of military personnel examined from the inception of the program to October 1, 1945, was 1153. On these persons a total of 2747 examinations, or an average of 2.4 per individual, was made. In addition 103 civilian navy employees and their families, interned in the Philippines during the Japanese occupation, were examined by us upon their return to the United States, an average of 2.8 examinations apiece being performed upon this group. A careful check was made upon the diagnoses of the military personnel, to ascertain whether the group examined was weighted upon the side of those with intestinal complaints, or established diagnosis of parasitism. As a series of fecal examinations on each patient is a routine procedure on many of the hospital wards, it was considered probable that our series would represent a random selection of the patient personnel. Such was found to be the case.

The examinations consisted of wet smear, zinc-sulfate concentrate, and iron-haematoxylin stain, carried out as described in an earlier paper by one of us (4). Cultures for *Endamoeba histolytica* were not a routine procedure, but were often employed as a check-up after treatment, using St. John's medium (1). Diagnosis of *Strongyloides* infection was usually made from the haematoxylin-stained fecal film, and Baermann concentrates (2), were utilized to follow the progress of treatment. No attempt was made to determine the actual incidence of enterobiasis among the military personnel, by means of anal swabs, and the two cases found in this series were diagnosed from adult worms in the feces. A series of examinations for pin-worm was performed on each of the children in the internee group, with negative results.

The percentages of the various intestinal parasites found in our examinations of the 1153 naval and marine patients, and also the "Theoretical Percent Infection" for the various amoebae, as derived from the figures given by Sawitz

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² The authors wish to express their appreciation to Lt. Alan C. Pipkin, who instigated the amoebic culture work, and to Ens. William C. Hill, who did much of the work in connection with the examination of the civilian internees.

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and Faust (5), is given in Table no. I. Of the group, 464, or 40.3 per cent, were infected with one or more parasites.

The repatriated civilians examined included both men and women, with a few children. They were a very homogeneous group, having for the most part been at Cavite Naval Base, near Manila, before the invasion, and were almost all interned at Santo Tomas University in Manila. A few were at Los Baños,

TABLE I

PARASITE	DEMONSTRATED PER CENT INFECTION	THEORETICAL PER CENT INFECTION
Hookworm.....	5.46	
<i>Strongyloides stercoralis</i>	0.78	
<i>Ascaris lumbricoides</i>	0.09	
<i>Trichuris trichiura</i>	1.87	
<i>Enterobius vermicularis</i>	0.17	
<i>Hymenolepis nana</i>	0.26	
<i>Taenia saginata</i>	0.17	
<i>Isospora hominis</i>	0.17	
<i>Giardia lamblia</i>	3.21	
<i>Endamoeba histolytica</i>	5.55	8.67
<i>Endamoeba coli</i>	17.61	23.17
<i>Endolimax nana</i>	20.64	27.17
<i>Iodamoeba bütschlii</i>	1.13	1.49
<i>Dientamoeba fragilis</i>	0.09	
<i>Chilomastix mesnili</i>	0.43	

TABLE II

PARASITE	DEMONSTRATED PER CENT INFECTION	THEORETICAL PER CENT INFECTION
Hookworm.....	8.73	
<i>Strongyloides stercoralis</i>	1.94	
<i>Ascaris lumbricoides</i>	7.76	
<i>Trichuris trichiura</i>	44.66	
<i>Isospora hominis</i>	4.85	
<i>Giardia lamblia</i>	9.70	
<i>Endamoeba histolytica</i>	27.18	38.28
<i>Endamoeba coli</i>	26.21	31.96
<i>Endolimax nana</i>	35.92	43.80
<i>Iodamoeba bütschlii</i>	3.88	4.73
<i>Chilomastix mesnili</i>	2.91	

Bilibid, or other prisons, during part or all of the Japanese occupation. Their parasite rate was high, eighty-one persons in the group having one or more species of intestinal parasite, as shown in Table no. II.

While Sawitz and Faust (5) give the probable percentages of amoebic infections demonstrable on the first, second, third and subsequent examinations (using an iron-haematoxylin stain and Faust's zinc-sulfate flotation method),

Faust *et al.* (3) in a comparison of various concentration and other techniques, find that a single zinc-sulfate flotation will suffice to demonstrate even quite light cases of hookworm infection. However, their series was small (five infections in 189 specimens), and in ours, in which most of the infections were also light, not all positives were diagnosed with a single concentration. Thus, in sixty-three cases of hookworm infection, fifty-four (or 85.7 per cent) were positive on the first examination, but seven (or 11.1 per cent) were diagnosed only on a second fecal examination, and the remaining two infections were found on the fourth and seventh examinations respectively.

It will be noted that the infection rate for *Dientamoeba fragilis* among the military personnel was extremely low, only one case having been found among all those examined. This does not compare well with a rate of 1.61 per cent found among naval personnel in the South Pacific (Markell, 4), and is presumed to be due to the difficulties experienced in securing absolutely fresh specimens for examination. The incidence of other parasites found in this series was generally lower than in the South Pacific (hookworm being 8.46 per cent, *Trichuris* 2.12 per cent, *E. histolytica* 8.09 per cent, etc., in the former series), but if it is assumed that the South Pacific rates reflect a considerable percentage of infections acquired in that area, this is to be expected. A large number of these patients had not seen service in the Pacific area, and some, of course, had not been overseas at all. *Giardia* infections, on the other hand, were encountered much more frequently than in the former study, where most of the personnel examined had been on atabrine suppressive treatment for malaria.

Two cases of *Isospora hominis* infection were found in the military personnel examined (again a lower rate than in the Pacific series, where five cases were found in 1371 patients), and five cases among the civilian internees. None of these patients had diarrhoea or dysentery, such as has been occasionally noted in *Isospora* infections.

The civilian internees, as mentioned above, form a most homogeneous group, and while the parasite rates shown are probably representative of one large internment camp, Santo Tomas University, it would not be safe to assume that parasite rates among other groups of internees or prisoners-of-war in the Philippines would be necessarily comparable.

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DDT DUST FOR THE CONTROL OF HEAD LICE^{1, 2}

FRANK A. COWAN, TED MCGREGOR AND NEAL M. RANDOLPH

Many requests, especially from school officials and civic organizations, have been made to the Texas State Department of Health concerning head louse control. A detailed report upon our experience, including methods of application, amount of DDT dust applied, and effectiveness of the insecticide, is given in this paper.

In addition to being a pest, the head louse, *Pediculus humanus var capitis* de Greer, has also been found capable of transmitting important diseases such as typhus and trench fevers. Various insecticides, including many home remedies, have been used for treating infested persons with varying degrees of success. These treatments in the main have been messy and have required repeated applications. Dusting with DDT powder appears to be an easier and more effective treatment.

In February, 1946, arrangements were made through the Hidalgo County Health Officer and officials of the Roosevelt School, McAllen, Texas, and the Saint Joseph School, Donna, Texas, to treat some of the children enrolled in these schools. The majority of these children were members of large families and lived under crowded conditions. Only volunteers were used, since some of the parents objected to the program and some of the children refused treatment. No child having any skin abrasion or sores on the scalp was dusted although some having freshly oiled hair were treated. At no time during this study were any harmful effects noted from the use of DDT dust. The ages of the subjects ranged from 6 to 14 years. Due to their irregular attendance 66 of the 173 children treated received only one application. Seventy three children were untreated and used as controls.

The degree of infestation was determined by searching carefully among the hairs for live lice and viable nits. In questionable cases a fine-toothed comb was used to find the lice, and nits were removed for microscopic examination to determine if they had hatched or were dead or empty. After examination each case was (arbitrarily) classified as heavy, medium, light, or none.

A dust composed of 10 per cent DDT and 90 per cent pyrophyllite was the insecticide used. A pint jar with a perforated lid, containing a weighed amount of dust, was used as a shaker to apply the material. A towel was placed around the neck of the child and brought forward to cover the eyes and nostrils and to keep the dust off the clothes. A shake of dust was applied at the back of the neck, above each ear and on the top of the head, and then rubbed or patted into the hair so as to distribute the insecticide evenly over the entire scalp. The amount of dust applied varied from 2.5 to 5.0 grams, the larger quantity being used on girls with long hair.

¹ The authors wish to express their appreciation to Dr. Mary Walton, Director, Hidalgo County Health Unit, for her assistance in this work.

² From the Texas State Department of Health, Austin, Texas.

Each subject treated was instructed not to wash his hair for one week. At the end of two weeks a second application of dust was made to all subjects present in school on that date, and a second examination made to determine the effects of the insecticide on the infestation. A third examination was made at the end of four weeks.

The results of this study are shown in the accompanying table. On the first examination, of the total of 246 children, 57 (or 23 per cent) had no infestation; 100 (or 41 per cent) had light infestation; 54 (or 22 per cent) had medium infestation; while 35 (or 14 per cent) had an infestation classified as heavy. No doubt constant washing and other home care had decreased the number of heavy cases. After the fourth week only one of the 173 treated children had any lice. This child had a heavy initial infestation and informed the writers that he had

TABLE I

NUMBER CHILDREN	NO. OF TREATMENTS	INITIAL INFESTATION				INFESTATION AFTER 4 WEEKS			
		Heavy	Medium	Light	None	Heavy	Medium	Light	None
66	1	10	9	23	24			1	65
107	2	19	27	48	13				107
73	none	6	18	29	20	6	23	31	13
Total...246		35	54	100	57				

washed his hair the day following the treatment. Many of the treated children had nits on the hair but these were far from the scalp and microscopic examination showed them to be empty. While it is usually suggested that two dust applications be made there was very little difference in control between the group that received one dusting and the group that had two applications of dust. In both groups the control was complete. In the check group the infestation remained fairly constant during the period of the study.

RECOMMENDATIONS

1. 10 per cent DDT dust is a safe and effective control for head lice when left on the hair for at least a week.

2. One level tablespoonful (3 to 5 grams of DDT powder) is adequate for treating a child's head if allowed to remain on the hair for at least one week.

THE TREATMENT OF CARRION'S DISEASE WITH LARGE TRANSFUSIONS^{1,2}

CORRIN H. HODGSON

Carrion's disease (Oroya fever, or verruga peruana) is a disease limited geographically to certain ravines and valleys of the Andes Mountains, principally in Peru, to a limited extent in Ecuador and Colombia and possibly in Bolivia and Chile. The causative organism is *Bartonella bacilliformis*. According to present knowledge, this organism is transmitted to man by the bite of a night-flying insect, *Phlebotomus verrucarum*.

There are two phases of clinical importance in this disease. The first phase of the illness may consist of a period of rather mild symptoms, including moderate fever, general malaise, headache and pain in the bones. The patient may make an uneventful recovery from this phase or a very serious illness due to severe anemia may develop. It is believed that the organism directly invades the erythrocytes and eventually causes a rupture of each cell invaded. In the severe anemic form, the organism can be readily identified microscopically on an ordinary blood smear stained with Wright's stain (fig. 1). The mortality rate in this anemic form is not exactly known, but it is at least 20 per cent and most likely much higher. This first phase of the illness is termed the "Oroya fever" phase.

If the patient recovers from this first phase of the illness, he remains apparently well for a period of a few weeks or two or three years. Then there develops the typical cutaneous eruption, consisting of varying numbers of small wartlike nodules in the skin (fig. 2). These cutaneous nodules may persist for a few weeks or a few months and ultimately disappear. Apparently in all cases the eruption develops sooner or later. It was formerly believed that the severe anemic form, Oroya fever, was a different illness from the eruptive form, verruga peruana. It is now known that they are two different stages of the same illness. The disease probably will not become important as far as the United States is concerned, except for an occasional traveler who may visit these endemic regions. Its importance to Peru should not be underestimated, as there are extensive regions of economic, historic and scenic importance where previously uninfected persons dare not remain at night.

The following two cases of Carrion's disease were the first in which the patients were treated successfully by repeated large transfusions. Small or infrequent transfusions have been used heretofore, but never in sufficient quantity to attempt to replace the amount of blood being destroyed. It is obvious from a study of these two cases that small amounts of blood would be valueless. Because this method is new and because it is contrary to the prevailing medical opinion in Peru, these cases are presented as a possible advance in the method

¹ Work done in the British American Hospital, Lima, Peru.

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of handling this highly fatal disease. It is hoped that further investigation of this method will be stimulated by this report.]



FIG. 1. (Case 1.) BLOOD SMEAR (WRIGHT'S STAIN) SHOWING *BARTONELLA BACILLIFORMIS* INVADING ERYTHROCYTES



FIG. 2. TYPICAL CUTANEOUS ERUPTION OF PERUVIAN VERRUGA

REPORT OF CASES

Case 1. A fifty year old white man of Peruvian nationality was admitted to the hospital April 4, 1942. His past history was negative except for malaria in 1914, fracture of the leg in 1917, and left inguinal herniotomy in 1938. He had been in the Valley of Huailas, Peru, where Carrion's disease is known to be endemic, in November and December, 1941, and in January, 1942, for several

days each time. He had stayed overnight in the cities of Carhuas, Huaraz and Caras during these trips. His present illness began on March 21, 1942, two weeks before admission or two months after the last possible exposure to the disease. The onset was sudden, with a chill followed by fever. He had had a maximal daily temperature of 39.5°C. (103.1°F.) with a return to normal each day. He complained on admission of sharp pain in the splenic region on deep inspiration, pain on pressure over the liver, a nonproductive cough, fever, malaise, weakness, and pain in the bones, especially those of the legs. He had been given nine tablets of quinacrine hydrochloride (atabrine) and two injections for malaria prior to coming to the hospital, although smears for malaria had been negative. Additional symptoms of which he complained at some time or other during his illness were: sensations of heat and cold, sweating, anorexia, restlessness, nervousness, soreness of the mouth and throat and hyperacidity.

The results of physical examination on admission were as follows: He appeared acutely but not severely ill. His height was 1.63 meters (64 inches) and his weight 54 kg. (119 pounds). He was by nature slender, nervous and apprehensive. His temperature on admission was 36.8°C. (98.2°F.), pulse 78 and respirations 20. The examination gave negative results except in the following respects: there was slight icterus of the skin and sclerae; he was weak; a slight friction rub, which was synchronous with the heart beat, was heard over the apex of the heart; blood pressure was 140 mm. of mercury systolic and 80 diastolic; the lungs were clear; the liver was 2 cm. below the costal margin and was slightly tender; there was tenderness in the region of the spleen but the spleen was not palpable at any time during his stay in the hospital. There were a large right inguinal hernia and a left inguinal herniotomy scar.

A smear for malaria was ordered and the laboratory technician, Srta. Manuela Solano, reported the smear negative for malaria but made the correct diagnosis by reporting "numerous endoglobular bodies of Barton."

Clinical course: Though the patient's illness did not appear severe on admission, within forty-eight hours his condition became much more serious. He became weak and pale and said he felt very poorly. The number of erythrocytes per cubic millimeter of blood had declined from a reasonable normal of 4,300,000 on admission April 4, to 1,370,000 on April 6, and the concentration of hemoglobin had dropped from 72 per cent to 32 per cent. Therefore, on April 6, a program of rather large transfusions was begun, and he received a total of 3,410 c.c. of citrated blood in six different indirect slow drip transfusions from April 6 to April 19, an average of approximately 570 c.c. each. One of the donors had had the disease about ten years previously. Except for a slight sensation of suffocation during one transfusion, there was no reaction to any of them. This same sensation was present on frequent occasions not associated with transfusions. One interval of six days and another of four days without transfusion were occasioned by the patient's family being persuaded by their friends that this was not proper treatment. However, when his condition would become more grave, the patient and the family were quite willing that he have more blood. There could be no doubt in the mind of anyone observing him closely

that the transfusions were beneficial, although the effect was only temporary. Additional treatment consisted of supportive measures, including supplementary vitamins, sedatives and intravenously administered fluids.

Jaundice, which was slight on admission, was severe by the fourth day and began to diminish by the tenth day. However, there was still slight jaundice when the patient left the hospital. The jaundice and the level of serum bilirubin corresponded closely to the destruction of blood. Some improvement in his condition could be noted after the tenth day, but he continued weak, restless and uncomfortable until about the twenty-fourth day after admission. He got up out of bed on the twenty-sixth day and left the hospital on the thirty-second day, having been without fever for three days. At the time he left the hospital he weighed 45 kg. (99 pounds) and was extremely weak but definitely felt that he would get well. The first cutaneous eruption developed on the left leg the first week in July, 1942, approximately three and one half months after the onset of symptoms. No serious complications arose during the course of his illness. When seen on August 6, 1942, he weighed 55 kg. (121 pounds) and was feeling very well except for blurring of vision and spots in the visual field of the right eye. There were vitreous opacities in the right eye. At that time he had a great many warts typical of verruga peruana located on the scalp, right upper eyelid, forehead, face, neck, many on the scrotum, arms and legs, but only a few on the trunk. He was seen again on December 23, 1942, when he felt perfectly well. His eruption had cleared entirely during November, approximately eight months after the onset of his illness, or five months after the first cutaneous eruption appeared. He was perfectly well when last heard from in 1944.

Case 2. A forty-three year old white man of Yugoslav birth was admitted to the hospital December 31, 1943. The past history was negative except for bubonic plague in 1907 and tuberculosis of the knee in 1926. He had stayed in the ravine of Huanchay, Peru, from October 26 to November 22, 1943, and again from December 6 to December 14, 1943. This is at an altitude of approximately 2,300 meters (7,500 feet) and is known to be "verruca country." He was taken ill December 8 with general malaise, insomnia, anorexia, fever, and pain in the entire body, particularly in the neck and arms, and such severe pain in both tibiae that he could not walk. On December 14 he came to Lima and took treatment for malaria. December 19 he bathed in the sea, thinking it might help him. Three hours later he had chills, high fever and aggravation of all his symptoms. On December 30 he first noticed jaundice, and came to the hospital the following day. In addition to the foregoing the symptoms of which he complained at some time or another during his illness were: nausea and vomiting, severe diarrhea for fifteen days, severe headache, nervousness, thirst and slight cough. He had no pain in the liver or spleen.

The physical examination on admission gave negative results except as follows: He appeared severely ill, anemic, and had jaundice of the skin and sclerae, grade 4 (on the basis of 1 to 4, in which 1 designates the mildest and 4 the most severe condition); rapid respiration at times; herpes simplex of the lips; a few marginal râles at the base of the left lung posterior; abdominal distention, grade 1+; the

liver was 3 cm. below the costal margin and slightly tender; the spleen was not palpable on admission but became definitely palpable one to two weeks later, reached a level 2 cm. below the costal margin, and was still palpable when the patient left the hospital. His weight was 70 kg. (154 pounds), height 1.73 meters (68 inches). His temperature on admission was 37.5°C. (99.5°F.) and during the illness varied from normal to a maximum of 39.1°C. (102.4°F.).

His first blood examination, taken shortly after admission gave the following results: concentration of hemoglobin 30 per cent, erythrocyte count 1,800,000 and leukocyte count 16,150 per cubic millimeter of blood. The urine contained 3.10 gm. of albumin per liter, and many granular and leukocyte casts. The diagnosis of his condition was correctly made by Srta. Manuela Solano, who reported "abundant endoglobular bodies of Barton" in the blood smear.

Clinical course: A program of large transfusions was begun immediately and between December 31, 1943, and February 1, 1944, the patient was given 8,150 c.c. of citrated blood by the slow drip indirect method in fifteen different transfusions. This is an average of approximately 550 c.c. for each transfusion. There was no reaction following any transfusion. None of the donors had previously had verruga. When this program was begun, he felt some improvement in his general condition. Profiting by my experience with the previous case, I did not wait until the concentration of hemoglobin in his blood dropped nor until he felt bad before giving more blood. Consequently, he did not revert to a state of extreme discomfort within a day or two after receiving blood, as the first patient did, but rather felt relatively comfortable throughout the illness. In other words, I was able to keep him in a state of relative, though not complete, comfort when his condition was compared to that prior to transfusions. By the sixteenth day after admission he was much improved, his blood was near normal, there were very few Bartonella in the erythrocytes, and he had not had a transfusion for six days. This prompted the hopeful progress note: "Patient making very satisfactory and prompt recovery. Now feels quite well." However, it was soon obvious that he had not yet recovered. The concentration of hemoglobin later declined, numerous Bartonella returned to the cells and it was necessary to give five more transfusions. Nevertheless, his general condition did not become serious during this relapse. Relapses of this nature are common in this disease. Additional treatment consisted of supportive measures including supplementary vitamins, sedatives and intravenously administered fluids.

He got up out of bed on the fortieth day after admission, and left the hospital on the forty-fifth day (February 15) feeling "perfectly well." He was not even weak. He was seen again in May, 1944. He said that he felt very well and had had no illness. He complained of an occasional mild pain in the shoulders and legs. His general appearance was normal. The concentration of hemoglobin was 79 per cent, erythrocytes numbered 4,100,000 and leukocytes 6,850 per cubic millimeter of blood. A blood smear showed no Bartonella. He reported again in November, 1944, at which time the typical cutaneous eruption of this disease was starting. Except for the eruption, he was in good health. It is interesting

to note that in this case the eruption developed nearly one year after the onset of his illness.

COMMENT

The treatment of Carrion's disease by transfusion is not new. Even fairly large transfusions have been given heretofore, but to my knowledge, this disease has not been treated with frequent, large transfusions in such quantity as may be necessary to replace the blood being destroyed, nor am I aware of such a large quantity of blood having been given to any one patient. It is in this aspect of the treatment that I believe hope lies for the present.

In the first case, the transfusions were necessarily interrupted. This gave an opportunity to observe the length of time beneficial effects would last. Relief, though striking, lasted for only thirty-six to forty-eight hours. In the second case, the transfusions were continued and, so far as possible, the concentration of hemoglobin was not allowed to decrease before more blood was given. The patient's reaction to the treatment was definitely much better than in case 1, he remained relatively comfortable throughout his illness, and the anemia was much better controlled.

Jaundice is a frequent complication of the anemic form of the disease. This is due to erythrocyte destruction. The jaundice will coincide with the amount of destruction of blood and is independent of the transfusions.

All the transfusions in these two cases were given by the slow drip, indirect, citrate method. This is preferred to the direct method, as it is difficult to give large quantities by the direct method and it is preferable to give the blood slowly over a longer period. I allowed the blood to drip at the rate of 60 to 80 drops per minute, taking approximately two to four hours for each transfusion. I do not believe that any statement as to the quantity of blood necessary can be made. This should be determined by the reaction of the patient and by the blood laboratory determinations. I am convinced that the blood should be given in sufficient quantities to keep the patient comfortable, to replace the blood being destroyed and to build the patient's blood up to normal or maintain it as nearly normal as possible. Judging from the reaction in case 1, this would require 500 c.c. to 700 c.c. or more daily during the more serious part of his illness.

As neither of these patients suffered from any severe complications, I had no opportunity to observe the effect of the transfusions under these circumstances. It would seem particularly important to maintain a fairly normal blood level in the presence of complications such as pneumonia. Perhaps the transfusions were of some benefit in preventing complications in these two cases.

One very valid objection to this treatment is the difficulty of obtaining such large quantities of blood. I believe that if this method should prove a definitely lifesaving measure in this disease some means should and would be found to provide blood for any one who may be unfortunate enough to contract the disease. Some of the donors in these two cases were professional donors. However, the great majority of them were friends of the patients and were very willing to contribute their blood.

Naturally, one cannot say what would have been the outcome if these two patients had not been treated in this manner. They are not presented as a representative series. I can merely state that they were both seriously ill, both had severe anemia, both were definitely improved by transfusions and both recovered.

No definite conclusion can be drawn from such a small number of cases as to the effect of this treatment on the mortality rate. The results, however, are certainly encouraging and the method should be given further trial. I feel that this method of treatment may constitute an advance in the battle on Carrion's disease and may prove a definite aid until specific treatment is developed. The results in these two cases would tend to cast doubt on the prevailing opinion that transfusions are harmful to patients who have Carrion's disease.

This form of therapy is not intended to be specific for Oroya fever. One could only expect that it would protect the patient from complications due directly to the severe anemia and thereby lower the mortality rate.

Recently Merino (1) reported favorable results in the treatment of two patients with Carrion's disease by the use of penicillin. This is more encouraging than other observations on the use of penicillin in Bartonellosis. In general, the antibiotics have not been proved to be efficacious against *Bartonella bacilliformis*. However, it is well established that penicillin is effective in preventing and treating secondary infections, such as pneumonia, which may complicate Oroya fever and cause the patient's death. Therefore it would seem to be a very logical procedure to administer penicillin throughout the more serious phase of Oroya fever in an effort to prevent secondary infection. To use it in this way would be similar to the present successful treatment of agranulocytosis where penicillin protects the patient from fatal secondary infection until his own defenses have recuperated. To my knowledge this has not been done with Oroya fever patients nor have I had an opportunity to try it. It should be investigated by all means. It is quite probable that large transfusions to counteract the anemia plus penicillin to protect the patient against secondary infection may appreciably reduce the mortality rate in this highly fatal disease.

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THE EVALUATION OF THE SURGICAL TREATMENT OF RECURRENT ECHINOCOCCIC CYSTS OF THE LIVER FOLLOWED BY DEEP X-RAY THERAPY¹

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In 1928, Dr. John F. X. Jones operated upon a patient for echinococcic cyst of the liver. Five years later, we operated for a recurrence. At operation, it appeared that the cyst involved about one sixth of the liver substance.

After removing a large number of daughter cysts and necrotic material, the cyst was marsupialized by sewing the cut edges to the peritoneum. The cyst was then packed with five iodoform gauze packs, each pack being five yards long and two inches wide. The gauze packs were allowed to remain undisturbed for a period of three weeks and were removed at the rate of about a foot daily.

Six months after the operation, daughter cysts were being extruded from the sinus. Since this was the second recurrence, we decided to try deep x-ray therapy. We based this on the known fact that eggs lose their fertility after x-ray treatment. We also knew that if a hen were x-rayed, the eggs would not be fertile.

Between 4/3/35 and 4/23/35 ten x-ray treatments using 200 K.V.P. with a filter of $\frac{1}{2}$ m.m. copper and 1 m.m. aluminum were given over the anterior surface of the liver totaling 1200 R. Between 4/25/35 and 6/4/35, nine treatments were given over the posterior surface of the liver totaling 1030 R. Two weeks following the second treatment, no cysts were extruded from the sinus. A week later the sinus healed.

The patient was seen at frequent intervals and nine years later, there was no evidence of recurrence.

We had hoped that other cases of echinococcic cysts would come under our observation but to date, we have not had another case. We realize that one good result does not mean that we have found a cure. We are simply reporting this case because of the possibility of echinococcic disease being brought back to this country by some of our troops who have been exposed to this disease. We thought deep x-ray therapy treatment might be of value in these cases. It certainly should be considered, at least.

¹ From Service of American Oncologic Hospital.



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IMMUNIZATION OF DUCKS AGAINST MALARIA BY MEANS OF KILLED PARASITES WITH OR WITHOUT ADJUVANTS¹

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Attempts to produce immunity against malaria in birds by the injection of killed malaria parasites have almost always failed. Gingrich (1) and Jacobs (2) however obtained results suggesting that protection against malarial infection in birds can be produced. Gingrich using *P. cathemerium* in canaries injected excessive numbers of heat or formalin killed parasites intravenously and challenged the birds two days after vaccination. Although evidence of protection was obtained, such a procedure could have little or no practical application. Jacobs employing *P. lophurae* in white Pekin ducks combined killed parasites with staphylococcus toxoid and used this preparation as a vaccine. Four of six ducks injected with this vaccine appeared to be protected as compared with non-vaccinated control ducks. Two ducks showed little if any protection, one died with high parasitemia whereas the other survived after high parasitemia. The birds were challenged in this experiment on the third day after the last of five injections of vaccine.

In an earlier paper from this laboratory (3) we reported that it is possible to protect adult ducks against malaria caused by *P. lophurae* by three injections of vaccine given four weeks apart. The vaccine was made of formalin killed *P. lophurae* parasites suspended in saline and incorporated by means of an emulsifying agent into a water-in-oil emulsion with paraffin oil in which killed, dried tubercle bacilli were suspended. The vaccinated ducks showed low parasitemia as compared with non-vaccinated control ducks and seven of eight of the vaccinated birds survived in contrast to a 50% mortality in the control birds. This report extends the previous experiments and presents new data on *P. lophurae* infection in normal ducks as well as further immunization studies with plain vaccines and vaccines containing adjuvants. Observations on immunization with *P. cathemerium* in ducks and cross immunity experiments are also presented.

MATERIAL AND METHODS

A strain of *P. lophurae* obtained from Dr. H. B. Van Dyke of Squibb Institute for Medical Research was used with the white Pekin duck as host. The strain was maintained by repeated passage through baby ducklings. Young ducks

¹ This study was aided by a grant from the John and Mary R. Markle Foundation.

² Surgeon (R), U. S. Public Health Service.

about two months old, weighing 2500 to 3000 grams were used for immunization. Although ducklings are more susceptible to malaria caused by *P. lophurae*, it was essential to use older ducks for immunization because the plan of the experiment required a period of at least two months from the first dose of vaccine to challenge. Furthermore, the capacity to be immunized and the susceptibility to infection appeared to change rapidly during the first few weeks of life.

Preparation of the Vaccine

Heavily parasitized blood was collected in citrate or heparin solutions by bleeding adult ducks from the jugular vein. The plasma was removed; the cells were washed twice with 0.85% saline solution, suspended in salt solution containing 0.10% formalin; the amount of formalin-salt solution was equal to that of the plasma. The cell suspension was kept at 4°C. for 18 hours. The cell-parasite mass was then washed three times with 0.85% saline solution. After the last washing, the supernate was removed. No attempt was made to separate the parasitized from the non-parasitized red cells; the entire cell-parasite mass was used as antigen. During the early part of the present study, large aliquots of the cell-parasite mass, after treatment with formalin, were injected intravenously into ducklings. The material proved to be non-infectious.

In some experiments the vaccine consisted only of cell-parasite mass suspended in saline; in others the antigen was combined with adjuvants. In the latter experiments, the saline suspension of antigen was mixed with an emulsifying agent, Falba³ and paraffin oil, "Bayol F"⁴ containing killed and dried tubercle bacilli. Such emulsions were prepared as follows: two volumes of the saline suspension of antigen with known number of parasites were added drop by drop to one volume of autoclaved and melted Falba and mixed with an emulsifying mixture were added two volumes of autoclaved paraffin oil of light viscosity containing killed and dried human type of tubercle bacilli, "Jamaica 22". The tubercle bacilli were killed by heat at 100°C. for 30 minutes and dried in vacuo over phosphorus pentoxide before being suspended in paraffin oil.

Vaccines prepared with these adjuvants are water-in-oil emulsions and show on microscopic examination (figure 1) parasitized and unparasitized red blood cells in minute water droplets surrounded by a menstruum of oil and Falba. The water droplets contain from one to several red cells and most of the tubercle bacilli are in the oil phase. Such emulsions are stable for long periods of time at or below room temperature. On gentle shaking, some of the oil rises to the top forming a layer of clear oil. The composition and amount of the vaccine used are described in the individual experiments.

In three experiments (1, 2, and 6) the vaccine was injected into the muscles of the chest, and in two (3 and 4), into the subcutaneous, fatty tissue of the groins and back of neck, and in one experiment, both the subcutaneous and intra-

³ Manufactured by Pfaltz and Bauer, Inc., New York, N. Y.

⁴ Bayol F is a paraffin oil of light viscosity obtained through the courtesy of Mr. K.L. Patterson of Stanco Distributors, Inc., New York, N. Y.

muscular routes were used. The general plan of immunization was to administer two or three doses of vaccine from four to six weeks apart, and to challenge the ducks four weeks after the last dose of vaccine.

The ducks were infected with from one to five billion *P. lophurae* parasites injected into a web vein. The challenge dose was selected by determining the minimum number of parasites which produced the highest mortality in ducks weighing from 2500 to 3000 grams.

Parasite counts, red cell counts and hemoglobin determinations were made daily during parasitemia and for varying periods thereafter from venous blood obtained from a leg vein. Parasitemia is expressed as the number of parasites per 100 RBC. This value was obtained by counting at least 500 cells.

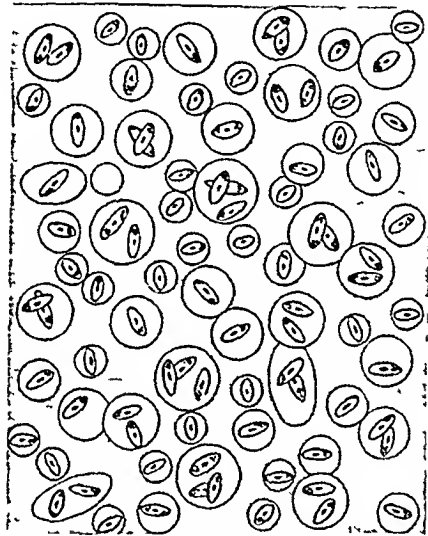


FIG. 1. SCHEMATIC DRAWING OF A WATER-IN-OIL EMULSION CONTAINING PARASITIZED RED CELLS

The cells are in the water droplets.

Hemoglobin content was determined in a photoelectric colorimeter by the alkaline hematin method and expressed in grams of hemoglobin per 100 cc. of blood (human). Red cell counts were made on hemocytometers calibrated by the Bureau of Standards.

Ducks surviving challenge were observed for several months. All the birds were eventually autopsied. In addition to routine pathologic examinations the sites of vaccine deposition were carefully noted and histologic sections of these were studied.

Course of P. Lophurae Infection in Normal Ducks

The strain of *P. lophurae* was maintained by repeated passage through ducklings less than two weeks old. The infecting doses of from one to two billion parasites used were fatal for the ducklings.

The course of *P. lophurae* infection in adult ducks weighing from 2500 to 3000

TABLE 1
P. lophurae infection in unimmunized adult ducks

INFECTING DOSE OF PARASITES	NUMBER OF DUCKS	PATENT PERIOD DAYS	PEAK: PARASITES/100 RBC	DIED		KILLED		SURVIVED
				0	2	0	1	
10 million	4	2 to 6	4 to 24	0	2	0	1	4
100 million	4	0 to 3	50 to 155	7	7	1	2	1
1 billion	16	0 to 2	37 to 133†	21	4	22	7	4
3 billion	47	0*	57 to 193‡			2	1	
5 billion	7	0	123 to 190§					

* 1 duck with 1 day of prepatency.

† 2 ducks 7% and 4%.

‡ 2 ducks with 3.2% and 3.3%; of the 19 ducks which died, one had 41.4%, one 74.8%, the rest had higher peaks.

§ 1 of 7 ducks had a peak parasitemia of 9%.

|| This figure may suggest that 4 of 26 ducks survived; it may be emphasized that the 22 ducks similarly infected and killed were exsanguinated to prepare antigen at a level of parasitemia which almost uniformly was followed by death.

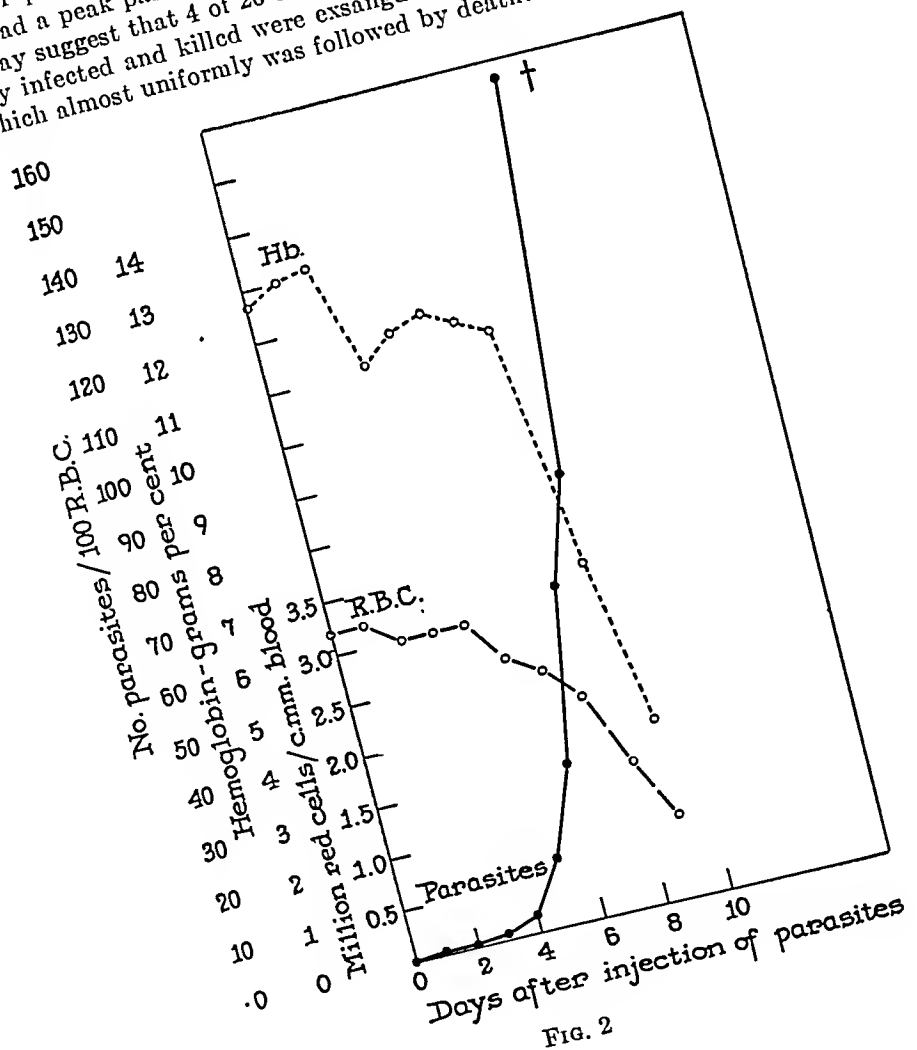


FIG. 2

grams is shown in table 1. Parasite counts, red cell counts and hemoglobin determinations were made daily from infection to death or recovery. Coincident with the development of parasitemia a sharp drop in red cell count and hemoglobin value developed. Death did not always occur at the time when maximum parasitemia was demonstrable in the peripheral blood; in some birds it occurred from one to two days later when the number of circulating parasites was diminishing. In such birds the red cell and hemoglobin values did not return to normal levels suggesting that acute anemia probably was the cause of death. At autopsy the unvaccinated ducks showed changes characteristic of acute malaria and anemia. When spontaneous recovery occurred, the red cell and hemoglobin values began to return toward normal levels within two or three days following the maximum drop in parasitemia. Typical examples are shown in figures 2, 3 and 4. In normal ducks responding to infection with low parasitemia, smaller changes occurred in the red cell and hemoglobin content of the peripheral blood.

IMMUNIZATION EXPERIMENTS

Experiments 1 and 2

The composition of vaccine and schedule of immunization in experiments 1 and 2 are shown in table 2. The vaccine was injected in divided doses of 0.5 to 1.0 ml. each in multiple sites in the muscles of the lateral aspects of the chest wall. Three injections of vaccine were given at approximately monthly intervals. Control and vaccinated ducks were challenged about one month after the last immunizing injection of vaccine by the intravenous injection of one billion parasites. The course of parasitemia in both immunized and control birds is shown in table 3 and figures 5 and 6 (in fig. 5, duck 27 omitted).

In experiment 1, three of the immunized ducks had a parasitemia of less than 1% for five days or less; one developed a parasitemia of 8% and died from causes other than malaria. All of the control ducks reached a parasitemia of 54% or more and two of them died from acute malaria. Of four immunized ducks in experiment 2, one reached parasitemia of 4%, one 3%, and one 0.2%, and the fourth showed no parasites on thin smear.

The duration of parasitemia in immunized ducks ranged from one to seven days. In contrast, of four control ducks, two died of acute malaria with high parasitemia, one developed a parasitemia of 37% which lasted for 18 days and in the fourth duck, the parasitemia rose to 15% on the 11th day but no parasites were found on the 20th day after infection.

Lesions of varying size and severity developed at the sites of injection. Following the first injection of vaccine nodular areas were found under the skin averaging in size about 20 x 20 x 5 mm. Following the second injection of vaccine, the lesions were larger, more indurated and, in six of eight birds, suppuration occurred. Because of the severity of the local reactions following the second dose of vaccine, tubercle bacilli were omitted from the third dose. Following injection of the third dose of vaccine indurated, non-suppurative lesions developed similar to those following the first dose of vaccine. The suppurating lesions

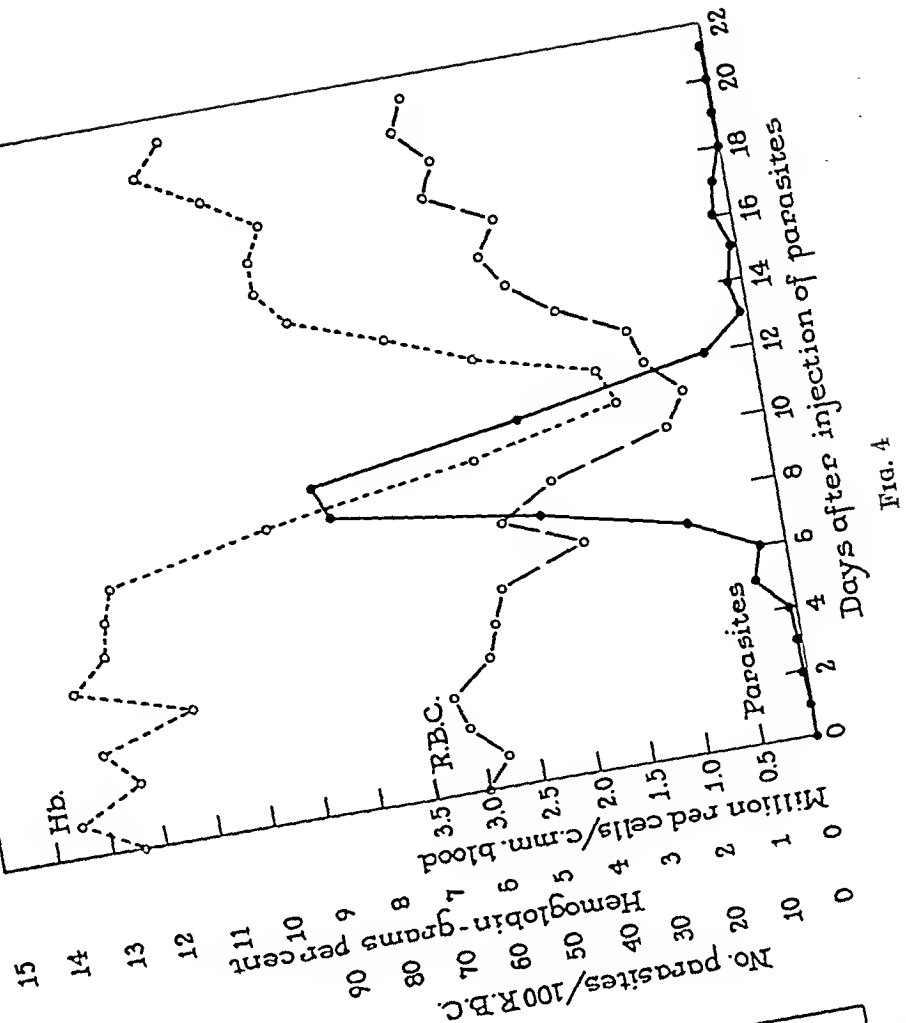


FIG. 3

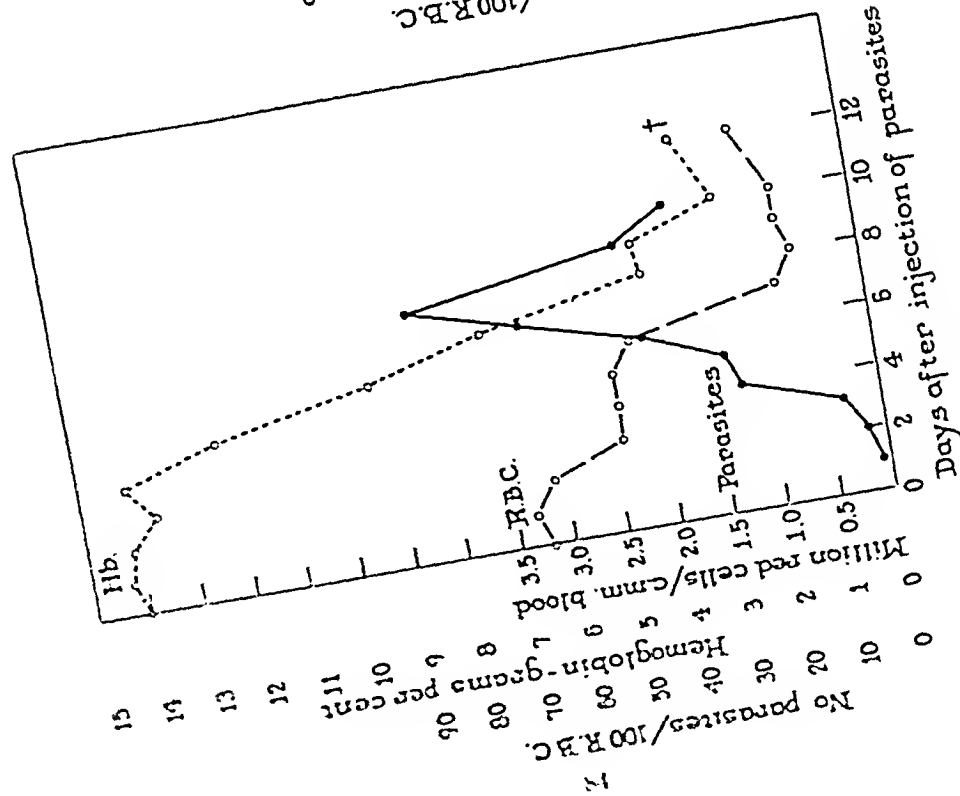


FIG. 4

TABLE 2
Composition of vaccine

EXPERIMENT	DOSE	TOTAL VOLUME	PARASITES	RED CELLS	FALBA	BAYOL F.	KILLED AND DRIED M. TUBERCULOSIS
		ml.	Billion	Billion	ml.	ml.	mg.
1	1	5	4.68	4.2	1.2	2.5	1.0
	2	12	20.8	22.4	4.0	4.0	0.156
	3	6	11.2	11.2	2.0	2.0	0
2	1	10	15.4	19.5	2.8	4.2	1.71
	2	8	11.8	11.8	2.7	2.7	0.10
	3	6	11.2	11.2	2.0	2.0	0

TABLE 3
Number of parasites per 100 red blood cells

GROUP	DUCK NO.	DAY AFTER INFECTION											
		1	2	3	4	5	6	7	8	9	10	11	12
Experiment 1													
Immunized	27	.2	.6	.8	1	5	8	Dead	(1)				
	34	0	.4	.6	.6	.2	.2	0	0	0	0	0	0 (2)
	28	0	.6	.2	0	0	0	0	0	0	0	0	0 (2)
	36	0	.4	0	.2	.2	0	0	0	0	0	0	0 (2)
Control	35	.2	.2	1	3	13	30	63	83	157	Dead		
	31	.4	0	2	3	9	10	24	58	93	77	Dead	
	37	0	.6	.4	1	6	4	16	42	80	83	49	8 (3)
	38	0	.2	1	2	3	3	10	30	30	54	28	6 (4)
Experiment 2													
Immunized	43	.2	.2	2	4	0	.4	0	0	0	0	0	0 (6)
	41	.4	1	3	—	1	.6	.4	0	0	0	—	0 (5)
	40	.2	0	0	0	0	0	0	0	0	0	0	0 (6)
	39	0	0	0	0	0	0	0	0	0	0	0	0 (6)
Control	44	.4	.2	1	5	8	21	59	102	128	98	Dead	
	47	.2	0	.8	4	4	21	34	76	88	108	Dead	
	48	0	.4	1	.8	2	2	2	5	16	22	37	22 (7)
	45	0	0	.8	1	.2	.6	.2	1	5	7	15	6 (8)

— Not done.

(1) 350 ml fluid in the peritoneal cavity.

(2) No parasites found through 23rd day.

(3) Became 0 on 18th day.

(4) Became 0 on 13th day.

(5) Duck 41 received only the first and second injections of vaccine and was infected weeks later. No parasites found through 41st day.

(6) No parasites found through 13th day.

(7) Became 0 on 20th day.

(8) Became 0 on 20th day.

which followed the second dose of vaccine healed spontaneously after several weeks.

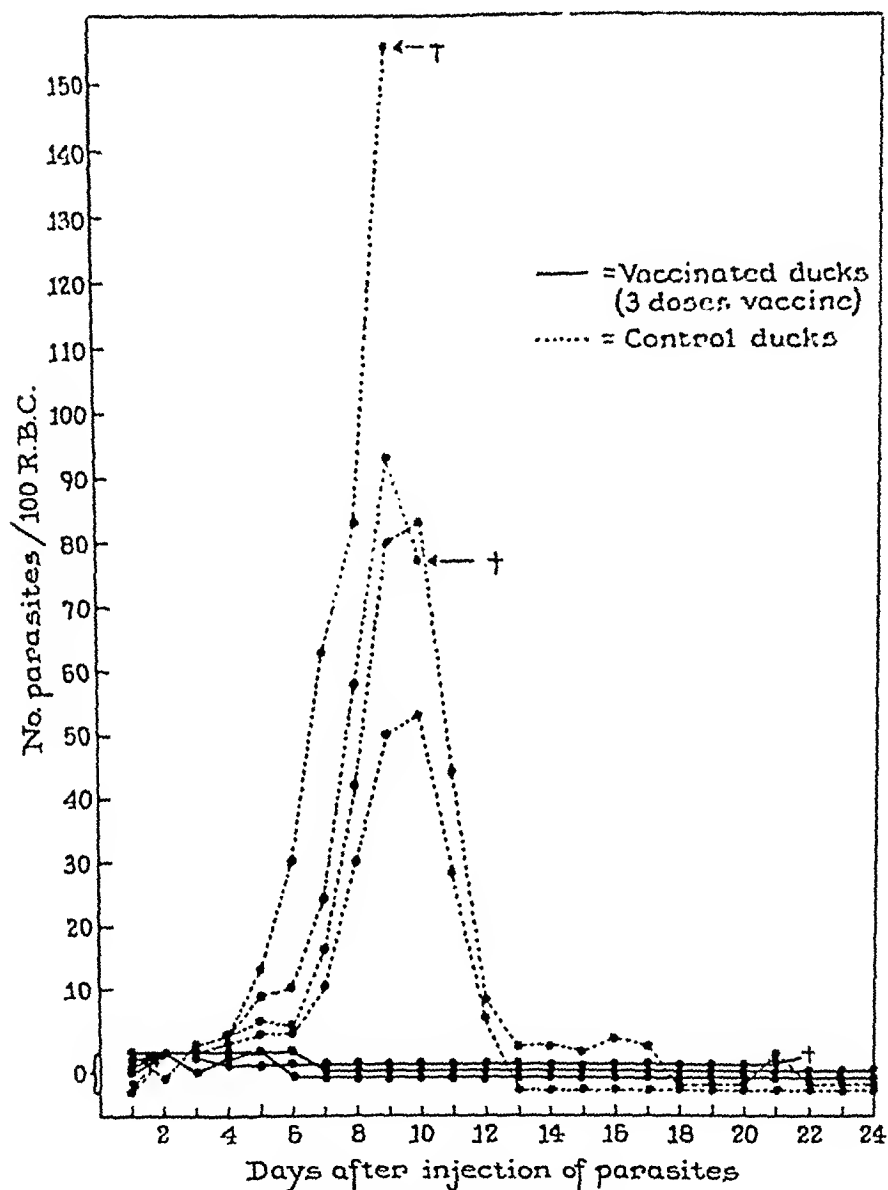


FIG. 5. EXP. I—PARASITEMIA

Experiments 3 and 4

Because of the severity of the lesions at the site of injection of vaccine in experiments 1 and 2, it seemed desirable to select a less reactive tissue for the site of injection of vaccine. Accordingly, in experiments 3 and 4, the vaccine was injected into the subcutaneous fat of the groins and the back of the neck.

Table 4 shows the composition of vaccine used in experiment 3. There were three groups of ducks; the first group received killed parasites suspended in saline emulsified in Falba and mineral oil containing killed tubercle bacilli;

the second group received a similar vaccine but without tubercle bacilli; the third group received killed parasites in saline. All birds were given a single dose of vaccine injected in multiple sites in the subcutaneous fat of the groins and back of the neck.

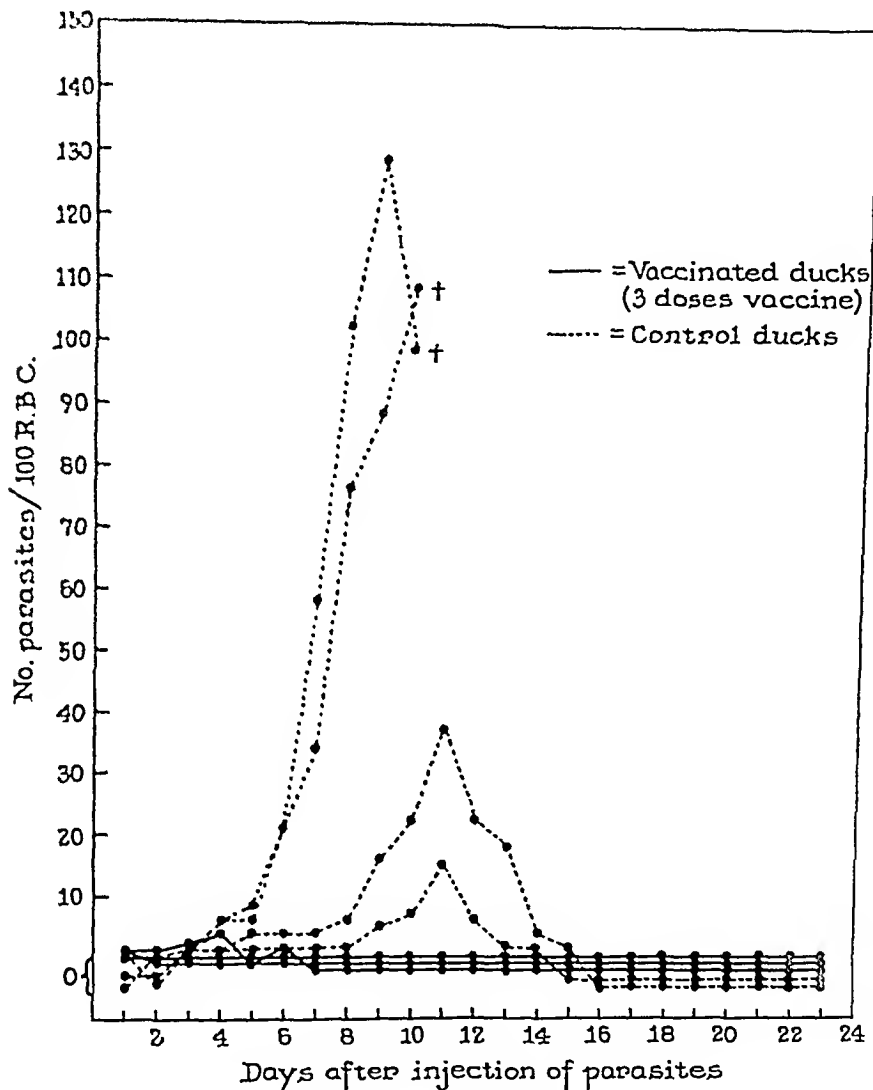


FIG. 6. EXP. 2—PARASITEMIA

The birds were challenged 50 days after the vaccination by the intravenous injection of one billion parasites. The progress of parasitemia in the vaccinated birds was similar to that in the controls. There was no evidence of protection in any of the groups of vaccinated birds.

Examination of the sites of injection of vaccine showed insignificant local lesions. At many sites, no masses could be seen or palpated, in others only slight subcutaneous thickening could be found. The skin was not involved.

Table 5 shows the composition of vaccine and schedule of injection in experiment 4. There were five groups in addition to the controls. The first received parasitized red cells in saline; the second was given parasitized red cells combined with Falba and mineral oil; the third was injected with parasitized red cells combined with Falba and mineral oil containing killed *M. phlei*; the fourth received

TABLE 4
Experiment 3
Composition of vaccine

GROUP	DOSE	TOTAL VOLUME	PARASITES	RED CELLS	SALT SOLUTION	FALBA	BAYOLF.	KILLED AND DRIED <i>M. TUBERCULOSIS</i>	
								mg.	
		ml.	Billion	Billion	ml.	ml.	ml.		
1	1	2.1	4.5	4.4	0	.42	.84	.042	
2	1	2.1	4.5	4.4	0	.42	.84	0	
3	1	2.1	4.5	4.4	1.26	0	0	0	

TABLE 5
Experiment 4
Composition of vaccine

GROUP	DOSE	TOTAL VOLUME	PARASITES	RED CELLS	SALT SOLUTION	FALBA	BAYOLF.	SESAME OIL	KILLED AND DRIED	
									<i>M. TUBER.</i>	<i>M. PHLEI</i>
		ml.	Billion	Billion	ml.	ml.	ml.	ml.	mg.	mg.
1	{1	2.25	5	5	1.35		.45	.9		
	{2	6.45	15	10.2	3.87		1.29	2.58		
2	{1	2.25	5	5		.45	.9			
	{2	6.45	15	10.2		1.29	2.58			
3	{1	2.25	5	5		.45	.9		.045	
	{2	6.45	15	10.2		1.29	2.58		.6	
4	{1	2.25	5	5		.45	.9		.045	
	{2	6.45	15	10.2		1.29	2.58		.6	
5	{1	2.25	5	5		.45	.9		.045	
	{2	6.45	15	10.2		1.29	2.58		.6	

parasitized red cells incorporated into Falba and mineral oil containing killed tubercle bacilli and the fifth received parasitized red blood cells incorporated with Falba and peanut oil containing killed tubercle bacilli. Two doses of vaccine were injected into the subcutaneous tissues eight weeks apart and the birds were challenged six weeks after the second dose of vaccine by the intravenous injection of three billion parasites. There was no evidence of protection in any of the vaccinated groups of ducks.

Examinations of the sites of vaccine injection revealed discrete nodular masses in the subcutaneous fat, unattached to the skin or fascia. The average size of such masses was 20 x 15 x 10 mm. At autopsy, the masses consisted of a thin, filamentous connective tissue capsule surrounding the injected vaccine which appeared grossly unchanged since it was injected.

Experiment 5

This experiment differs from all the others in this study in that the vaccine was administered both subcutaneously and intramuscularly. The original plan of the experiment to give two doses of vaccine subcutaneously was changed because the results of experiment 4 suggested that the subcutaneous fat was an unsuitable site. Accordingly, a third dose of vaccine containing three times more antigen was injected in the muscles of the chest in eight sites.

TABLE 6
Experiment 5
Composition of vaccine

GROUP	DOSE	TOTAL VOLUME	PARASITES	RFD CELLS	FALBA	DAYOL F.	KILLED AND DRIED M. TUBERCULOSIS
		<i>ml.</i>	<i>Billion</i>	<i>Billion</i>	<i>ml.</i>	<i>ml.</i>	<i>mg.</i>
1	{ 1	2.75	5	4.25	.55	1.1	.055
	{ 2	3	4.9	4.6	.6	1.2	.06
	{ 3	10.25	15	14.1	2.05	4.1	8.2
2	{ 1	2.75	.	5	.55	1.1	.055
	{ 2	3	.	5.3	.6	1.2	.06
	{ 3	10.25	.	10	2.05	4.1	8.2

There were two major groups of vaccinated birds; one received parasitized red cells incorporated into Falba and mineral oil containing killed tubercle bacilli; the other was identical except that normal duck red cells were used in place of parasitized red cells. The groups were further subdivided so that half of the birds received the first two doses of vaccine in one site; the other half received the first two doses in three sites. In both groups the third dose of vaccine was given intramuscularly into eight sites. The composition of the vaccine and schedule of vaccination are shown in table 6.

A challenge dose of three billion parasites was given intravenously forty days after the first dose of vaccine. The four non-immunized control ducks died of acute malaria; three of them from eight to nine days, the fourth, thirteen days after challenge. Five ducks injected on three separate occasions in three, three and eight sites respectively with killed parasites, Falba and mineral oil containing killed tubercle bacilli survived to be challenged. Two died within twenty-four hours after challenge. In the third duck, a parasitemia of 18.4% was reached on the sixth day after challenge and the bird was found dead on the following day. Another duck showed maximum parasitemia of 52.8% on the seventh day;

the parasitemia decreased to 19.4% on the day of death, which was the ninth after challenge. In the fifth duck, a parasitemia of 11.2% was reached on the sixth day, then the number of parasites decreased to 8.6%. This duck was found dead on the ninth day.

Three ducks receiving the same vaccine described above but injected in one, one and eight sites survived to be challenged. One duck in which the parasitemia rose slowly reaching 16.8% on the tenth day was found dead on the eleventh day. In another duck there was low-grade parasitemia reaching 9.2% on the tenth day, falling to 6.8% on the twelfth day, death occurring on the fourth day; the duck was found dead on the eleventh day.

Five ducks injected with normal duck red blood cells incorporated with Falba and mineral oil containing killed tubercle bacilli injected on three different occasions in three, three and eight sites survived to be challenged. Three of these ducks showed maximum parasitemias of 93.2%, 126% and 100.8% respectively and all died on the ninth day. A fourth duck ran a low-grade parasitemia which lasted nineteen days but never exceeded 1%. This duck recovered. The fifth duck had a low grade parasitemia not exceeding 1% until the tenth day after which it rose to 23.4% on the fifteenth day. The blood became negative on the seventeenth day and remained so as long as observed through the twenty-sixth day.

In summary, the four control ducks died of malaria with high parasitemia. Not including the two vaccinated ducks that died the day following challenge, two of the remaining six that received parasites combined with Falba and mineral oil containing killed tubercle bacilli died of malaria with parasitemias of 52.8% and 60% respectively. The other four ducks died with parasitemia from seven to fourteen days after challenge but the maximum parasitemia in these ducks reached 18.4%, 11.2%, 16.8% and 10.4% respectively. In two of these ducks the parasitemia was diminishing before death. Of the five ducks injected with normal duck red blood cells incorporated with Falba and mineral oil containing killed tubercle bacilli, three behaved as unvaccinated ducks, one after low-grade parasitemia lasting nineteen days, the other after prolonged parasitemia which reached its peak on the fifteenth day and disappeared on the seventeenth day.

Examination of the sites of vaccination showed local lesions similar to those already described; namely, where the vaccine was injected subcutaneously, small focal lesions with thin walls surrounding unchanged vaccine were found; where the vaccine was injected intramuscularly, the lesions were larger, more diffuse, and there was considerable fibrous tissue proliferation about tiny pockets of oily vaccine. No suppuration occurred at the site of injection of vaccine in any birds in this experiment.

At autopsy, the ducks receiving vaccine containing Falba and mineral oil in which killed tubercle bacilli were suspended showed large amounts of fluid in the peritoneal cavity and markedly abnormal livers. The livers weighed from

114 to 185 grams; a from two to threefold increase over the weight of normal livers. They had a peculiar yellow color and the cut surfaces had a homogeneous, waxy appearance suggesting amyloidosis. Histological sections of the liver and spleen stained with hematoxylin and eosin or congo red showed the characteristic picture of amyloidosis (see plate 1).

In view of the abnormal findings in the livers and peritoneal cavities of these birds, it is difficult to interpret the significance of the differences in parasitemia between the normal and vaccinated ducks. The latter group showed in four of



PLATE 1. LIVER OF A DUCK INJECTED WITH KILLED PARASITES AND KILLED TUBERCLE BACILLI IN PARAFFIN OIL SHOWING EXTENSIVE AMYLOIDOSIS

the six ducks which survived beyond the first day of challenge a rather prolonged and low-grade parasitemia followed by death. It was not determined whether the low-grade parasitemia was evidence of incomplete protection of immunological nature or was connected with the abnormalities in the liver and elsewhere or was due to some other cause. The cause of the unexpected deaths in two vaccinated ducks during the first twenty-four hours after challenge is not known. Both these ducks at autopsy showed liver changes of the type just described but no other obvious cause for death.

Experiment 6

In this experiment, like in experiments 1 and 2, the vaccine was injected into the muscles of the breast. The composition of vaccine and schedule of injections are summarized in table 7. There were two large groups of birds, one receiving killed parasites in saline emulsified in Falba and mineral oil containing killed tubercle bacilli, the other receiving the same number of killed parasites suspended in saline. The ducks of the first group were subdivided into two subgroups, one receiving one injection of vaccine, the other a second injection of vaccine one month later. All the ducks receiving killed parasites in saline were given two doses of vaccine one month apart. The first challenge included only ducks receiving oil vaccine and was made six weeks after the second injection of vaccine in one half of the ducks and ten weeks after the first and only dose of vaccine in the other half. The remaining birds were subdivided again into groups to be challenged at three and six-month intervals.

TABLE 7
Experiment 6
Composition of vaccine

GROUP	DOSE	TOTAL VOLUME	PARASITES	RED CELLS	FALBA	BAYOL F.	SALT SOLUTION	KILLED AND DRIED M. TUBERCULOSIS
		<i>ml.</i>	<i>Billion</i>	<i>Billion</i>	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>	<i>mg.</i>
2	{ 1	11	15	13.5	2.2	4.4	0	1.98
	{ 2	4.7	14.9	10.6	.94	1.88	0	2.00
3	{ 1	11	15	13.5	0	0	6.6	0
	{ 2	4.7	14.9	10.6	0	0	2.8	0

This plan of vaccination and challenge resulted from the experiences obtained from experiments 1 through 5. From experiments 3, 4, and 5, it was evident that the subcutaneous tissue was not a satisfactory site of injection. The pathologic changes in the livers of birds receiving relatively large amounts of the oil adjuvant vaccine made it desirable to reduce the amount of vaccine and the number of injections. It also seemed desirable to test the intramuscular injections of saline vaccine to determine whether or not adjuvants were necessary. Finally, it seemed important to determine the duration of immunity produced by killed parasites.

Challenge 1

Five ducks that received two injections of vaccine containing killed parasites, Falba and mineral oil plus killed tubercle bacilli; five ducks receiving a single injection of such vaccine and five normal ducks of the same age and weight were challenged six weeks after the second dose of vaccine and ten weeks after the single dose of vaccine. The challenge dose was three billion parasites given intravenously.

Of five ducks receiving a single injection of vaccine, one died. All showed low-grade parasitemia with peaks of 5.6%, 2.2%, 2.0%, 1.6% and 0.8% respectively. Four of the five birds showed no circulating parasites after the tenth day; in the fifth duck parasites were not demonstrable after the thirteenth day.

Challenge 2

Challenge 2 was made eight weeks after the second injection of vaccine or eighteen weeks after the first (single) dose of vaccine. Included in this challenge

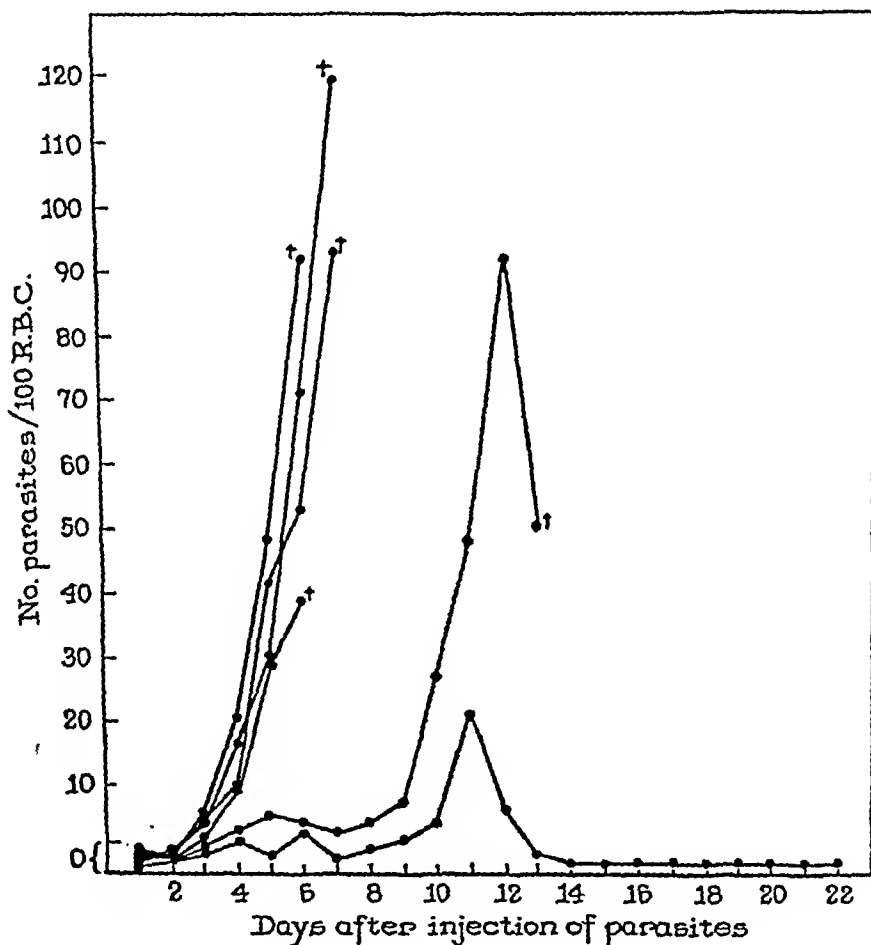


FIG. 8. EXP. 6—CHALLENGE 2 CONTROLS PARASITEMIA

were six non-vaccinated control ducks, five ducks that received two injections of vaccine containing killed parasites, Falba and Bayol with killed tubercle bacilli, five ducks that received one injection of the same vaccine and four ducks that received two doses of killed parasites in saline. The challenge dose was increased from three to five billion parasites because in challenge 1, there were two survivors in the non-vaccinated control ducks.

Of the six non-vaccinated controls, five died of acute malaria with high para-

sitemia (fig.8). In the sixth duck, at its peak the parasitemia was 23.8% on the eleventh day, following which it subsided rapidly and the blood became negative on the fifteenth day.

The result in the five ducks that received two injections of vaccine was as follows: One died with parasitemia of 6% on the fifth day of massive intra-peritoneal hemorrhage; one died of acute malaria on the ninth day with a parasitemia of 103.4% (fig. 9). The other three survived low-grade parasitemias

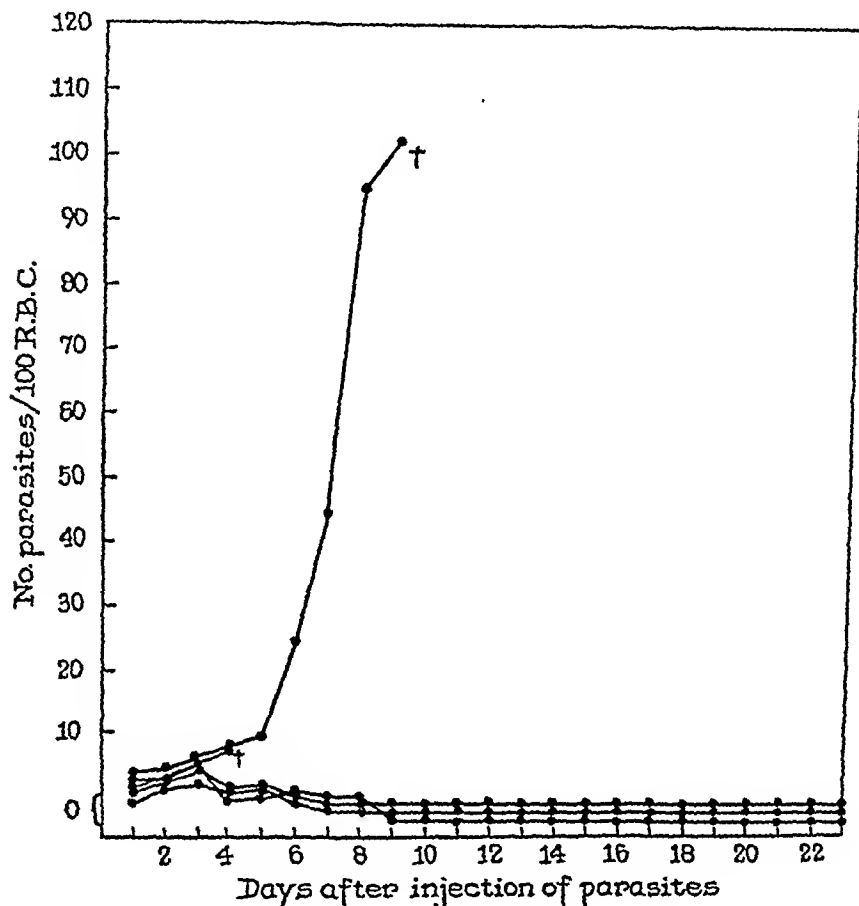


FIG. 9. EXP. 6—CHALLENGE 2 IMMUNIZED WITH ADJUVANTS 2 INJECTIONS OF VACCINE

which, at their maximum, reached 5.0%, 4.0% and 1.6% respectively. All the survivors in this group cleared their blood of parasites by the ninth day after infection.

Of five ducks that received one injection of vaccine containing adjuvants, four survived after parasitemia of varying degree, the peak parasitemia being 36.6%, 4.0%, 3.8%, 3.8% respectively (fig. 10). All four had negative blood smears on or before the ninth day. One duck in this group died on the second day after challenge of causes unrelated to acute malarial infection.

Four ducks vaccinated with two doses of killed parasites in saline were also challenged in this experiment (fig. 11). All showed parasitemia. One showed

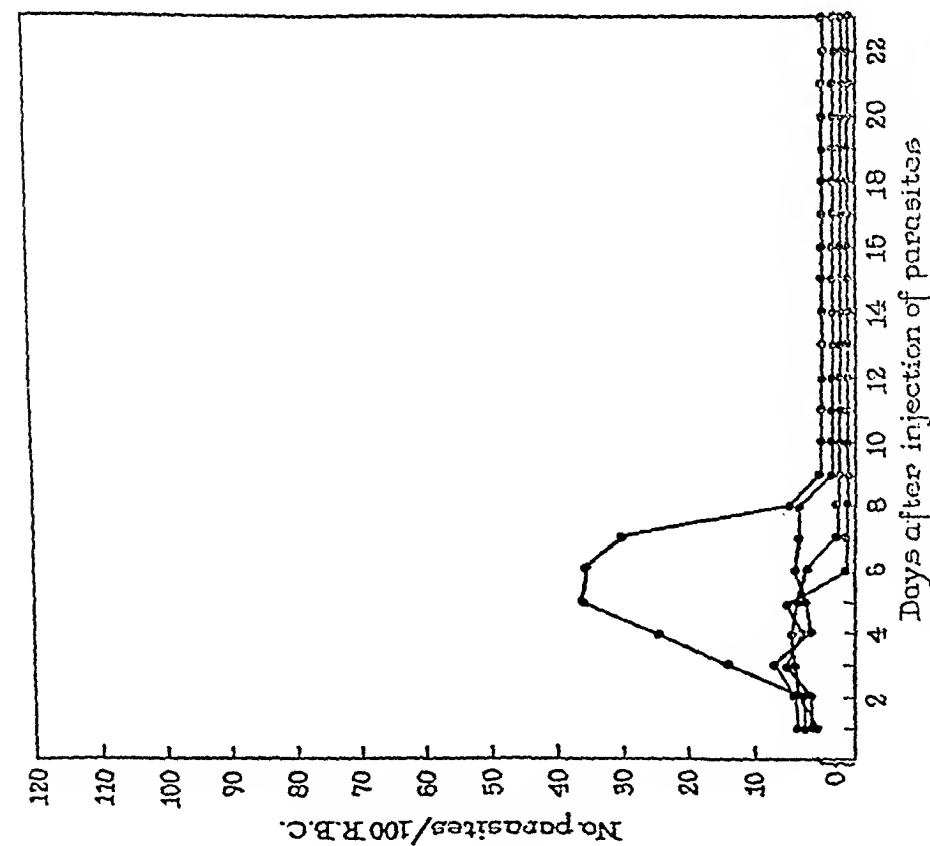


FIG. 10. EXP. 6—CHALLENGE 2 IMMUNIZED WITH ADJUVANTS 1 INJECTION OF VACCINE

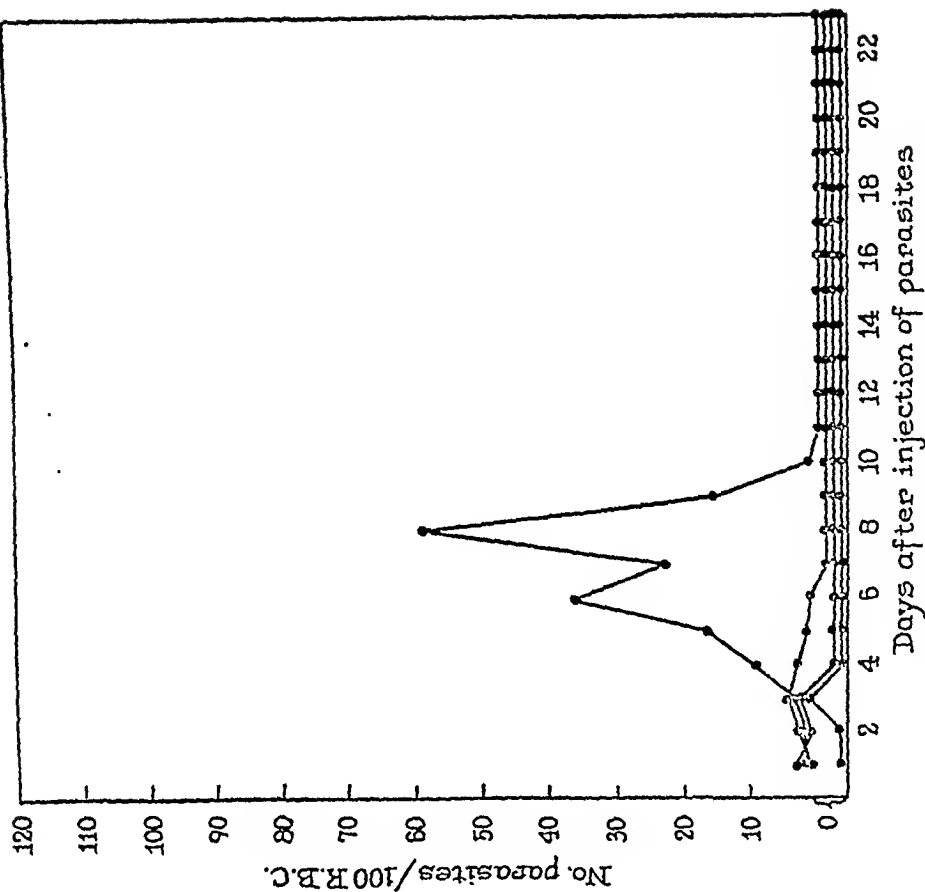


FIG. 11. EXP. 6—CHALLENGE 2 IMMUNIZATION WITHOUT ADJUVANTS 2 INJECTIONS OF VACCINE

the first group of ducks, on the eighth day, the other showed peak parasitemia of 93% on the 10th day. Parasitemia persisted in this group until the eleventh day in three ducks and until the thirteenth day in the fourth.

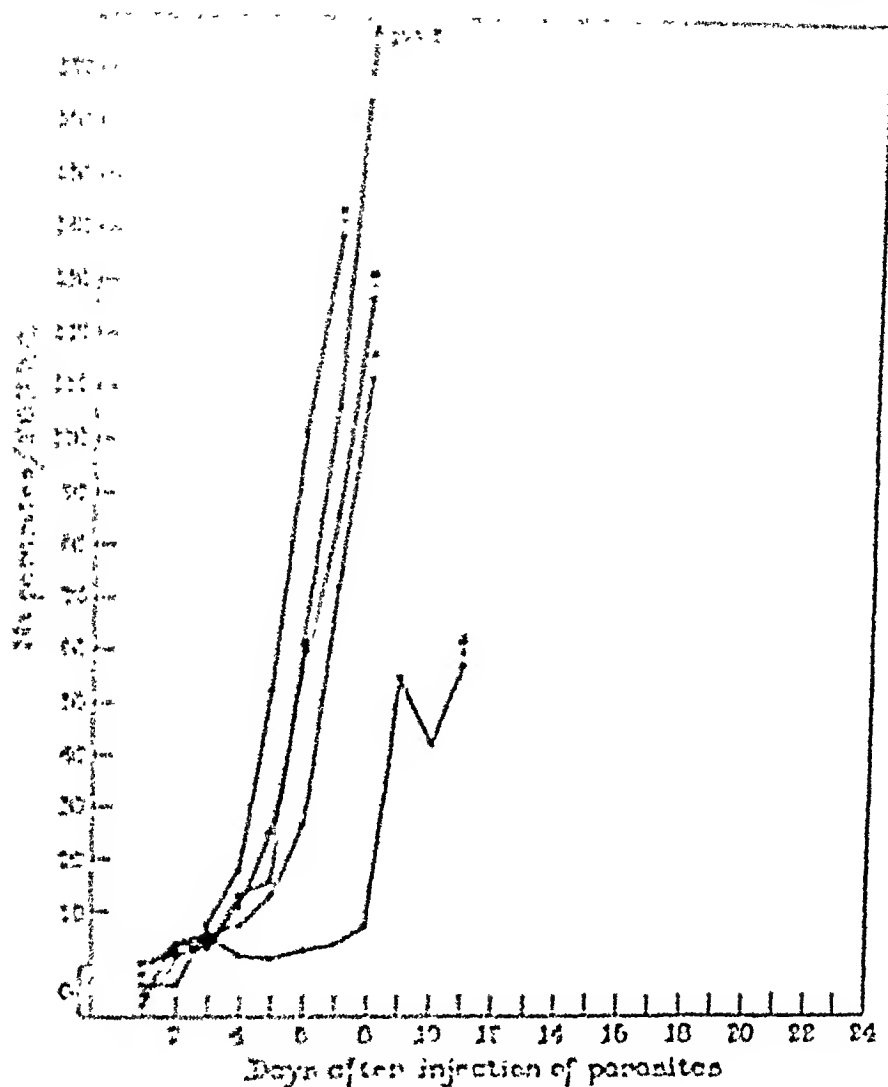


FIG. 12. Exp. 6—Challenge 3 Controls

Challenge 3

This group of ducks was challenged seven months after the administration of the first, and six months after the second dose of vaccine. The infecting dose was five billion parasites.

All of five controls died of acute malaria, four having parasitemia ranging from 111.6% to 193%, the fifth had a peak parasitemia of 57% (fig. 12).

Of four ducks that received two doses of vaccine containing adjuvants, one died of acute malaria with parasitemia of 81.6% (fig. 13). The other three

showed peak parasitemia of 22.6%, 10.0% and 6.2%. They cleared their bloods of parasites by the ninth day.

Five ducks were challenged that received one injection of vaccine containing adjuvants (fig. 14). One died a non-malaria death on the fourth day after challenge, the parasitemia being 2.8%. One died of acute malaria on the seventh day with a parasitemia of 109.2%. Three survived. At the peak of parasitemia, the counts were 25.4%, 6.8% and 1.6%. The peripheral blood of two

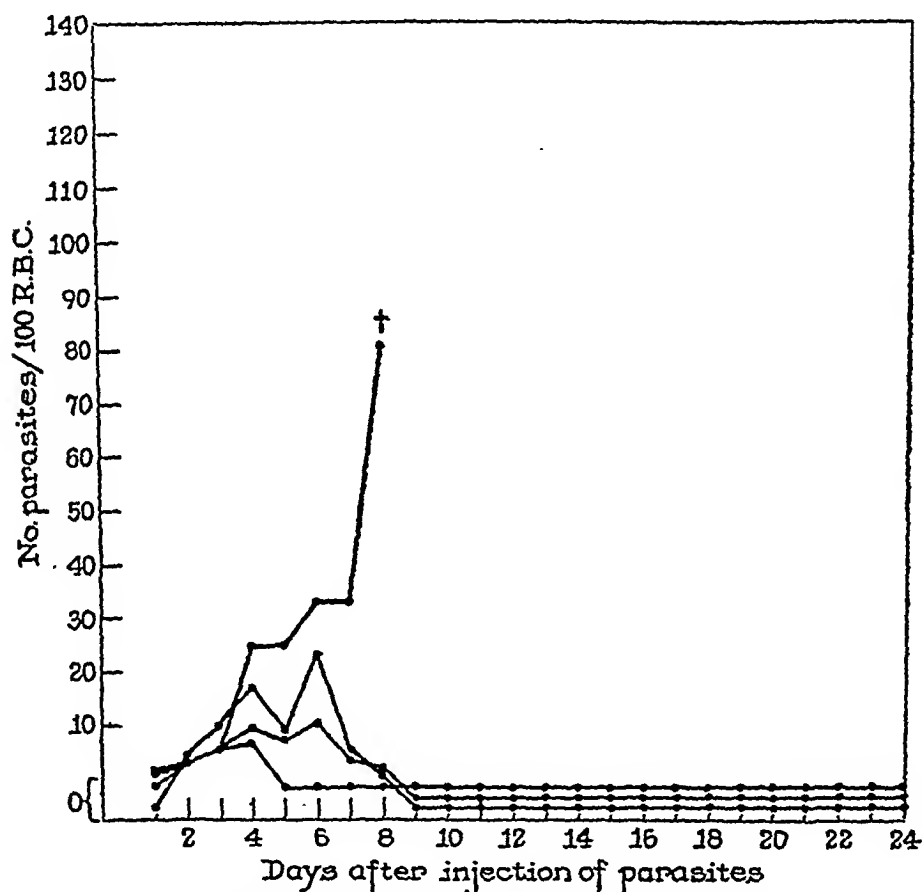


FIG. 13. EXP. 6—CHALLENGE 3 IMMUNIZED WITH ADJUVANTS
2 INJECTIONS OF VACCINE

became negative on the eighth day, the third ran a prolonged low-grade parasitemia lasting through the twenty-fourth day.

Of four ducks vaccinated with two doses of killed parasites in saline, two died of acute malaria with parasitemias of 140% and 79.2% on the seventh and eighth days respectively (fig. 15). The other two survived infections which were relatively severe. In one the parasitemia diminished rapidly and the blood became parasite-free on the eleventh day. The other duck showed a maximum parasitemia of 21.4% on the fifth day. This subsided rapidly, the blood being parasite free on the ninth day.

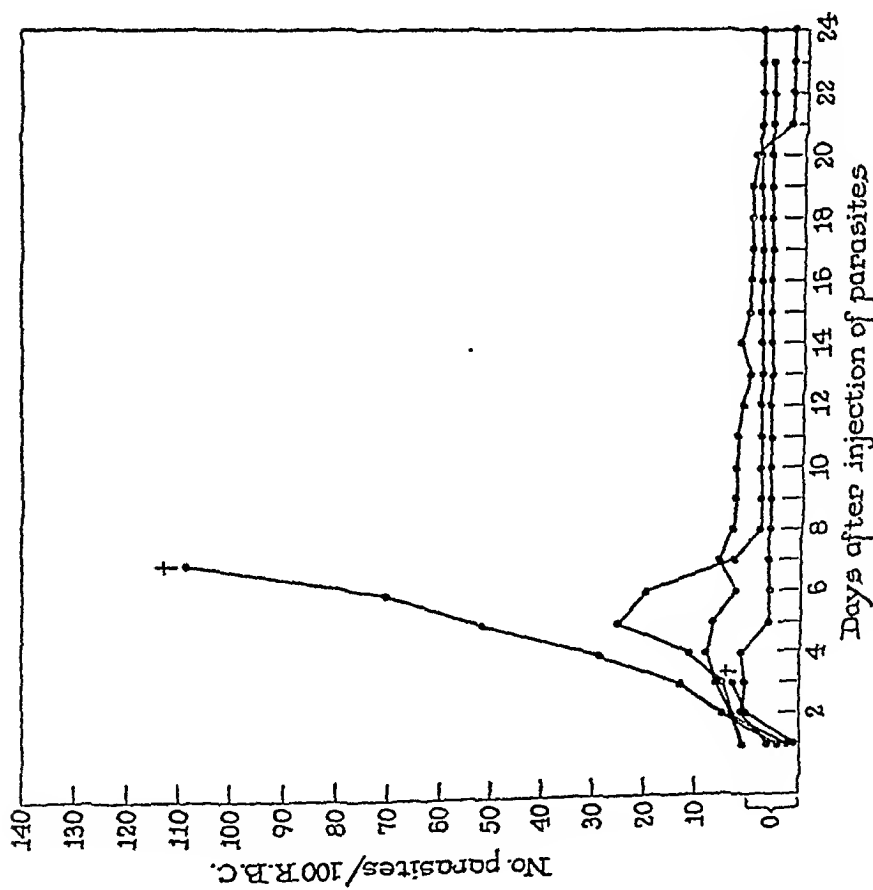


FIG. 14. EXP. 6—CHALLENGE 3 IMMUNIZED WITH ADJUVANTS
1 INJECTION OF VACCINE

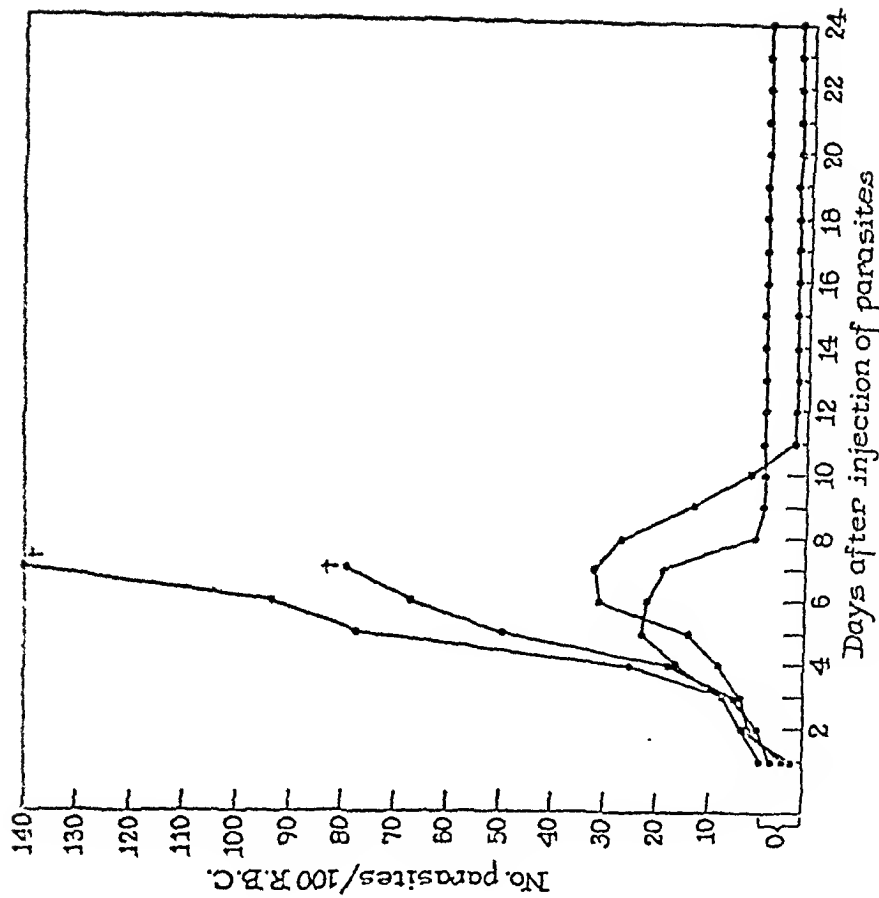


FIG. 15. EXP. 6—CHALLENGE 3 IMMUNIZED WITH PARASITES IN
SALINE. 2 INJECTIONS

Summary of experiment 6. Birds injected with killed parasites suspended in salt solution without adjuvants receiving two injections showed protection in challenge 2 i.e. fourteen weeks after the second and eighteen weeks after the first injection of vaccine; all of four survived infection, one had at its peak 58.4% and the other three birds only low-grade parasitemia. Another group of ducks vaccinated the same way and challenged seven months after the first injection of vaccine showed less protection since two of four ducks died from acute malaria and the two survivors had a maximal parasitemia of 32.4% and 21.4% respectively. These experiments indicate that ducks can be protected by killed parasites without adjuvants but the protection is of relatively short duration.

Ducks injected once with killed parasites and adjuvants, i.e. paraffin oil, Falba and killed tubercle bacilli, were challenged at three different intervals of time after vaccination. At ten weeks after vaccination, they were well protected since none died with malaria and all had only low-grade parasitemia, terminating from ten to thirteen days after infection. At eighteen weeks after vaccination, none of the ducks died with malaria; one showed a parasitemia of 36%, the others developed only low-grade parasitemia.

At seven months after vaccination, of four ducks injected with one dose of vaccine, one died of malaria, the surviving three showing a maximum parasitemia of 25.4%, 6.8% and 1.6%. The protection seen seven months after vaccination was less than seen at the shorter intervals after vaccination. Ducks vaccinated twice with parasites and adjuvants showed slightly more protection than those receiving a single injection. The difference was clearly evident at the time of the first challenge, i.e. 10 weeks after vaccination. Subsequent challenges showed little difference of protection between groups vaccinated once or twice.

Local reactions at the site of injection of vaccine

The injection of killed parasites without adjuvants caused no tissue reaction noticeable in the living bird. At autopsy, the findings varied with the time elapsed after vaccination. In ducks autopsied more than seven months after vaccination, little or no tissue reaction or vaccine was found at the sites of injection. In ducks autopsied in less than five months after vaccination, there were occasional deposits of vaccine surrounded by thin layers of connective tissue. Most sites of injections showed only slight pigmentation. At the sites of injections of parasites in water-in-oil emulsion containing killed tubercle bacilli, the amount of vaccine-residue and tissue reaction was conspicuous. The lesions were of two distinct types; they were either encapsulated masses containing vaccine or of infiltrative character. With the latter type, either the muscles were infiltrated with oily residue of vaccine or the fascia was thickened diffusely suggesting that the vaccine spread along fascial planes. In both types of lesions there was a conspicuous amount of connective tissue reaction about the vaccine-residue. These lesions did not involve the skin and there was no suppuration.

EXPERIMENT WITH *P. CATHEMERIUM*

Having found that ducks can be protected against *P. lophurae* infection by vaccinating them with formalin killed parasites with or without adjuvants, it seemed desirable to determine whether ducks could be immunized against infection with *P. cathemerium*. This species of plasmodium is less virulent for

TABLE 8
Composition of vaccine (*P. cathemerium*)

GROUP	DOSE	TOTAL VOLUME	PARASITES	RED CELLS	SALINE SOL.	YALDA	DAYOL F.	KILLED AND DRIED M. TUBERCULOSIS
		ml.	Billion	Billion	ml.	ml.	ml.	mg.
1	{1	16.0	9.2	26.4		3.2	6.4	1.8
	{2	13.5	10.0	26.4		2.7	5.4	1.0
2	{1	16.0	9.2	26.4	9.8			
	{2	13.5	10.0	26.4	9.8			

TABLE 9
Infection with *P. cathemerium* in unimmunized and immunized ducks

GROUP	DUCK	PATENT PERIOD DAYS	MAXIMUM PARASITEMIA	
			Days after challenge	Parasites per 100 RBC
Unimmunized	{1*	7	4	30.0
	{2	9	3	30.4
	{3	10	4	23.8
	{4	5	3	23.8
	{5	9	5	32.4
Immunized with adjuvants	{1	4	3	19.6
	{2	3	3	11.6
	{3	4	3	20.0
Immunized without adjuvants	{1	7	3	19.4
	{2	6	3	23.8
	{3	7	2	14.6
	{4	4	2	10.0
	{5	5	3	22.2

* Died on the 8th day after infection with 9.6 parasites per 100 RBC.

adult ducks than *P. lophurae* since most of the adult ducks infected with up to 7.5 billion parasites survive the infection after developing a parasitemia ranging from about 20% to 50%. The infection with *P. cathemerium* is characterized by parasitemia appearing within twenty-four hours after intravenous infection. The parasitemia rises rapidly and usually disappears from ten to fourteen days after infection.

The strain of *P. cathemerium* was obtained from Dr. Fruma Wolfson of the School of Public Health, Johns Hopkins University. It was maintained by repeated passage through baby ducklings. In doses of from one half to one billion, it killed ducklings regularly with parasitemias ranging from 31% to 76%.

Only one immunization experiment was done with formalin killed *P. cathemerium* parasites. The experiment was conducted in the same way as those with *P. lophurae*. One group of ducks was immunized with killed parasites suspended in saline; another group with the vaccine incorporated into oil containing killed tubercle bacilli. The dose and schedule of immunization, as well as the observations after challenge are given in tables 8 and 9. The ducks were challenged by the intravenous injection of 7.5 billion *P. cathemerium*.

The control ducks developed maximum parasitemia, ranging from 23.8% to 32.4% and their blood showed no parasites from six to thirteen days after infection.

The ducks immunized with killed parasites alone developed maximum parasitemias ranging from 10% to 23.8%. The maximum parasitemia was reached in a shorter time in the immunized birds. The parasites disappeared from the blood by the fifth to eighth days after infection. In the ducks immunized with parasites and adjuvants, the parasitemia reached 11.6% to 20.0% on the third day of infection. Parasites were not found in the blood after the fourth day of infection.

As indicated previously, survival of infection with *P. cathemerium* cannot be used as a criterion of resistance. The experiment showed, however, that in the vaccinated birds the maximum parasitemia tended to be slightly lower and was reached faster than in the controls. There was also a difference in the duration of parasitemia in the three groups of ducks; namely, the duration was shorter in the two immunized groups.

CROSS IMMUNITY EXPERIMENT

It seemed desirable to determine whether ducks surviving infection with *P. lophurae* are resistant to infection with *P. cathemerium*. Four adult ducks were infected with ten to one hundred millions of *P. lophurae*. They showed parasitemia of varying degree and survived the infection. One month after their bloods were free of parasites, they were reinfected with one billion *P. lophurae*. Three of the ducks had parasites (0.2% to 0.8%) in their blood for one or two days; the fourth duck had no detectable parasites in its blood. Six weeks later, the ducks were infected by the intravenous injection of 7.5 billion *P. cathemerium*. All of the four birds had parasites in their blood but parasitemia did not exceed 1.8% and the parasitemia lasted only for one to two days. A control duck infected the same way developed a parasitemia reaching 30.2%. Thus this experiment indicated that ducks surviving *P. lophurae* infection were protected against infection with *P. cathemerium*.

Red blood cell count, hemoglobin content of the blood during infections with P. lophurae and cathemerium in vaccinated ducks

Vaccinated ducks developing parasitemia showed a concomitant decrease in the number of red cells and hemoglobin content of the blood in a way similar to

that found in the non-vaccinated control birds. In ducks vaccinated and developing a low-grade parasitemia (of 1% or less) or no parasitemia, the red cell and hemoglobin values showed in some birds no change at all and, in some, a slight, and, in others, a considerable decrease, with prompt return to normal values. The decrease in the red cell count and hemoglobin values occurred, at the time, in relation to the inoculation, when parasitemia was progressive in other ducks belonging to the same group.

These observations suggest that the absence of parasites or the low parasitemia in vaccinated birds may be viewed not as an evidence of failure of the parasitization of the red cells but as a probable invasion of the cells by parasites and their prompt removal from the circulating blood. It may be recalled that in ducks vaccinated with normal duck red cells combined with paraffin oil, Falba and killed tubercle bacilli, the parasitemia following challenge was like that in the non-vaccinated birds. Thus it appears that the probable removal of parasitized erythrocytes in the immunized ducks may be a specific activity of the immune process. This inference is supported by observations in monkeys (Coggeshall and Kumm; Eaton (4)) showing that in immune monkeys the serum contains agglutinins as well as protective antibodies.

DISCUSSION

In contrast to widely held views, the above experiments show that it is possible to produce immunity against malaria parasites without infection. In challenging the ducks, a large number of parasites were injected. In the control ducks, infection resulted almost uniformly in high parasitemia but did not produce death in all birds. In the immunized ducks, the infection usually resulted in low-grade parasitemia and recovery; in some ducks parasites were not found in the blood. Another manifestation of immunity was the shortening of the duration of parasitemia. In the controls which survived, the parasitemia usually lasted for fifteen days while, in the immunized, it was terminated as a rule in about ten days. It may be noted that the immunity did not prevent infection in most of the ducks; however, attention may be called to the fact that with *P. lophurae* infection in the duck, a very large number of parasites, from one to five billion, were injected to produce lethal infection in the control birds.

The immunity was judged by survival, degree and duration of parasitemia. Failure to find parasites in the peripheral blood does not exclude the presence of parasites either in the blood or organs. Furthermore, it was found that in immunized birds there may occur without demonstrable or with low-grade parasitemia a drop, then a rise of the number of circulating red cells at the time when such change occurs in unvaccinated ducks with parasitemia. This observation suggests that the red blood cells are parasitized and the parasitized cells either do not reach the circulating blood or are rapidly removed from it. The factors presumably involved in the removal of the parasitized red cells were not elucidated upon but at least some of them appear to be immunologically specific since similar changes in the circulating red cells do not occur in ducks injected with non-parasitized red cells combined with the adjuvants. The absence of parasitized red cells in the peripheral blood of immunized ducks might be due to increased phagocytosis of parasites in the organs. The increased phagocytosis might be

brought about by specific humoral antibodies rather than by an increase in the capacity of the macrophages to phagocytize the parasites. The role of humoral antibodies in protection was shown by Coggeshall and Kumm (4) who succeeded in modifying the infection with *P. knowlesi* of rhesus monkeys by injection of serum from immune monkeys.

Immunization with homologous killed parasites was demonstrated not only against *P. lophurae* but also against *P. cathemerium*. Striking degree of cross immunity against *P. cathemerium* was found in ducks that survived repeated infection with *P. lophurae*. Cross protection was not tested against *P. lophurae* infection.

It was noted above that in experiment 5, amyloidosis was found in all the ducks immunized with vaccines combined with paraffin oil containing killed tubercle bacilli. In lesser degree, amyloidosis was present in the majority of ducks of other experiments receiving similar injections. It might be suggested that amyloidosis played a part in the resistance of immunized ducks. It might be pointed out, however, that amyloidosis did not occur after the injection of killed parasites suspended in saline without the adjuvants; nevertheless, protection was present. Subsequently, it was found (5) that, in ducks, amyloidosis is produced by a single injection of killed tubercle bacilli suspended in paraffin oil. It may be mentioned that in monkeys successfully immunized with killed *P. knowlesi* combined with paraffin oil and killed tubercle bacilli, amyloidosis did not occur (6).

CONCLUSIONS.

1. It is possible to immunize adult ducks against infection with *P. lophurae* or *P. cathemerium* by injection of formalin killed parasites combined with paraffin oil containing killed tubercle bacilli and an emulsifying agent. With *P. lophurae* infection, the immunity is manifested by prevention of death or by lowering the degree and shortening the duration of the parasitemia and, with *P. cathemerium*, by modifying the course of parasitemia.

2. Repeated intramuscular injections of killed *P. lophurae* combined with the adjuvants protect adult ducks for at least six months. One single intramuscular injection into multiple sites protects but it is less effective than repeated injections.

3. When the injections are made into the subcutaneous fat instead of the muscles no protection results.

4. Repeated intramuscular injections of killed *P. lophurae* suspended in saline solution immunize against the parasites but the protection is less effective than immunization with the aid of the adjuvants.

5. Repeated intramuscular injections of killed *P. cathemerium* either suspended in saline solution or combined with the adjuvants protect against *P. cathemerium* as judged by modification of parasitemia.

6. Unvaccinated ducks surviving repeated infections with *P. lophurae* showed conspicuous protection against *P. cathemerium* as evidenced by very low-grade parasitemia of very short duration.

7. There appears to be a relationship between protection and the local tissue reaction to the injected vaccine. The local reaction depends on the tissue into which the vaccine is deposited as well as on the presence of the adjuvants. When vaccine combined with the adjuvants is deposited into the fatty tissue, no protection follows and there is only very slight tissue reaction resulting in a thin, filamentous capsule and the vaccine has the gross appearance of recently deposited vaccine. When the vaccine without the adjuvants is introduced into fatty tissue, it disappears from there leaving only a slight pigmentation.

8. Normal or parasitized red blood cells in oil containing killed tubercle bacilli (as well as killed tubercle bacilli in oil), produce amyloidosis in the duck. Immunity against malaria does not depend on the presence of amyloidosis because it is possible to immunize ducks with killed parasites without killed tubercle bacilli and oil and without the production of amyloidosis.

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PREMATURE RUPTURE IN SCHIZOGONY; AN EXPLANATION FOR MULTIPLE INFECTION OF RED BLOOD CORPUSCLES WITH MALARIA PARASITES

S. G. MASILLAMINI¹

Multiple infection of red blood corpuscles is of common occurrence, particularly in heavy infections. It is much more common in malignant tertian than in benign tertian infections, while in the case of quarten infections it is very rare. Binary fission of the malaria plasmodia during their early development within the red blood cells has been recently offered as an explanation for the phenomenon of multiple infections.

Though binary fission is the normal method of reproduction in certain protozoa, spore-formation in schizogony has always been considered as the normal method of reproduction in the asexual phase of malaria plasmodia. A characteristic of reproduction is that it takes place in maturity and not in infancy, whether it is by equal division, unequal division or multiple division. Writing about reproduction of a cell, Calkins observes: "Ultimately its possibilities of further vitality as a single individual are exhausted and it undergoes its final manifestation of vitality. The significance of this final act is a function of all organisations whereby the organisation is further parcelled out to two or more trustees." Hence to imagine multiple infection is a result of binary fission of the malaria protozoa in their infancy is not in line with the biological principle just mentioned.

There is no well-defined knowledge about how the merozoites behave between their liberation and subsequent attachment to red blood cells. The merozoites after liberation "get themselves attached to the red blood corpuscles," "invade the red blood corpuscles", "penetrate into the red blood corpuscles", "are scattered and pass to infect fresh corpuscles"—this is how the behaviour has been variously described. There is an impression that the merozoite moves about of its own accord and selects the red blood corpuscle. We imagine that the merozoite has the power to select the red blood corpuscle when we say "it is contrary to biological laws for two or more parasites to attack the same erythrocyte and in so doing leave hundreds of other cells unparasitized." This may be true in the case of parasites that move about and have the power of selection. But there will be no question of violation of biological laws if we imagine the process to be, not one of active invasion, but one of passive mechanical deposition.

When the parasite is five or six hours old, it shows evidence of amoeboid activity. Activity during the merozoite stage has not been described. The active movement mentioned by Wenyon is only after attachment to the erythrocyte. He says merozoites attach themselves to red blood corpuscles and by active movements, similar to those shown by the sporozoites, make their way into the cells. It is not known whether this is actually an observation, or only

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an inference based on the analogy of the diagram of Schaudin illustrating the penetration of a red blood corpuscle by a sporozoite. The notion of penetration is bound up with the conception of the intra-cellular position of the parasite, accepted by many. Perhaps the merozoite is not active. It is only a spore and spores, like seeds, have not been observed to take part in propagation except passively. They get mechanically deposited and start growing. The amoeboid motility of the parasite practically disappears or is very sluggish at the end of 36 hours, even though sporulation commences as late as about 42 hours and 5 to 6 hours are taken for the process of schizogony. The merozoites that form during this resting stage have not been noticed to be motile. It is easy to believe that merozoites are not motile when they are discharged. If they are not motile the only process by which they get themselves attached to erythrocytes may be passive mechanical deposition, perhaps due to the differences in electric potential, helped on by the force of the blood current.

A fully mature schizont may be considered to be one in which the merozoites are fully developed and completely separated from one another and also from the debris formed during the process of schizogony. When such a fully developed schizont bursts, one would expect that the merozoites would get separated completely from one another unless they get entangled in the granular debris in their vicinity. Knowles (4) observed that the process of schizogony is really one of budding, so that one may come across schizonts in one portion of which merozoites have become fully differentiated, whilst in the other the individual merozoites have not yet been differentiated. In reproduction in nature, premature birth is of common occurrence, the causes at work being known in some cases and unknown in others. Therefore a premature rupture of a schizont will not be a strange event. When such a premature rupture takes place, the merozoites are not fully developed and they are not completely separated from one another. Some of them may still be attached to one another or shreds of cytoplasm may be linking them together. The debris might not have been fully separated out, so that the merozoites may be entangled in it when rupture takes place. If the merozoites are discharged in this state, naturally there will be every chance of two or more merozoites moving together and getting deposited on the same erythrocyte. Merozoites are known to move together in groups. This is perhaps how multiple infection of an erythrocyte takes place. This hypothesis of premature rupture of schizonts is offered as an explanation for multiple infection. It is not essential for purposes of this hypothesis to presume that the merozoites are non-motile. Even if they are motile, owing to the premature rupture, the attachments between the merozoites and the entanglements in the debris may be so strong that some of them may not be able to get away singly and therefore they get attached together to the erythrocyte.

To explain the phenomenon of multiple infection alone, it may not be necessary to presume premature rupture, because it is reasonable to expect that when there is a group of merozoites, there is every chance of two or more merozoites getting deposited on the same erythrocyte at the same time, since each individual merozoite is small compared with a corpuscle in the vicinity. It is a simultane-

ous invasion. It will be a question of random chance of deposition, between the number and size of the erythrocytes on the one hand and the number and size of the merozoites on the other, in the small space surrounding the groups of merozoites which have just burst from the schizont. The common occurrence of multiple infections in the case of *Plasmodium falciparum* may be for the reasons that 1) *Plasmodium falciparum* merozoites are the smallest (the merozoites of *Plasmodium falciparum* *quotidianum* are generally less than $0.5\ \mu$ in size), 2) Heavy infections of *Plasmodium falciparum* are more common, 3) Schizogony takes place in the internal organs and capillaries where there is crowding, 4) Infected cells in capillaries and internal organs have a tendency to clump together, more than in the case of *Plasmodium vivax* infections. But to explain (a) the supposed process of actual division going on in the ring stage and (b) the presence of fine filaments connecting the parasites, the hypothesis is useful.

Alassandrin had observed in fresh blood actual division going on. This division was supposed to be binary fission; but it can be explained by imagining that the division is only a continuation of the process of separation of the merozoites which should have been completed in the schizont itself but for the premature bursting. Opposed to this observation of Alassandrin is Wenyon's experience that he could not observe fission or conjugation. Craig who had believed in conjugation gave up the theory finally.

The presence of fine filaments joining the merozoites is due to the non-separation of the merozoites from one another completely. If the rupture takes place just immediately before maturity, the process of separation of the merozoites may continue even after they get deposited on the red blood corpuscle, and either the separation may be completed leaving behind no signs that the parasites were from the same schizont, or further separation may stop in the middle leaving behind cytoplasmic filaments connecting the parasites. But in case the bursting takes place considerably in advance of maturity, then the process of separation may not continue fully because the time has arrived for the process of growth to commence and therefore the process of division stops, and hence we find that even in fully grown double parasites there is often a filamental link between the two parasites.

Wenyon (6) observes that the interpretation of the phenomenon of multiple infection as an attempt at a primitive method of binary or multiple fission at a very early stage of development, is highly speculative.

All the appearances found in multiple infection can be explained on the supposition that the merozoites are not separated from one another completely when they get deposited together on erythrocytes, and in cases where no connecting strands can be seen it may be that the parasites were separated fully after deposition, or two or three individual merozoites were deposited simultaneously. The rings may run into one another or overlap, giving rise to many appearances for which binary fission has been offered as an explanation. The hypothesis suggested in this article will very well explain all these.

The appearances in the photo-micrographs in the articles on binary fission by Hingst (1) and Beach (2) can be explained with the help of this hypothesis.

an inference based on the analogy of the diagram of Schaudin illustrating the penetration of a red blood corpuscle by a sporozoite. The notion of penetration is bound up with the conception of the intra-cellular position of the parasite, accepted by many. Perhaps the merozoite is not active. It is only a spore and spores, like seeds, have not been observed to take part in propagation except passively. They get mechanically deposited and start growing. The amoeboid motility of the parasite practically disappears or is very sluggish at the end of 36 hours, even though sporulation commences as late as about 42 hours and 5 to 6 hours are taken for the process of schizogony. The merozoites that form during this resting stage have not been noticed to be motile. It is easy to believe that merozoites are not motile when they are discharged. If they are not motile the only process by which they get themselves attached to erythrocytes may be passive mechanical deposition, perhaps due to the differences in electric potential, helped on by the force of the blood current.

A fully mature schizont may be considered to be one in which the merozoites are fully developed and completely separated from one another and also from the debris formed during the process of schizogony. When such a fully developed schizont bursts, one would expect that the merozoites would get separated completely from one another unless they get entangled in the granular debris in their vicinity. Knowles (4) observed that the process of schizogony is really one of budding, so that one may come across schizonts in one portion of which merozoites have become fully differentiated, whilst in the other the individual merozoites have not yet been differentiated. In reproduction in nature, premature birth is of common occurrence, the causes at work being known in some cases and unknown in others. Therefore a premature rupture of a schizont will not be a strange event. When such a premature rupture takes place, the merozoites are not fully developed and they are not completely separated from one another. Some of them may still be attached to one another or shreds of cytoplasm may be linking them together. The debris might not have been fully separated out, so that the merozoites may be entangled in it when rupture takes place. If the merozoites are discharged in this state, naturally there will be every chance of two or more merozoites moving together and getting deposited on the same erythrocyte. Merozoites are known to move together in groups. This is perhaps how multiple infection of an erythrocyte takes place. This hypothesis of premature rupture of schizonts is offered as an explanation for multiple infection. It is not essential for purposes of this hypothesis to presume that the merozoites are non-motile. Even if they are motile, owing to the premature rupture, the attachments between the merozoites and the entanglements in the debris may be so strong that some of them may not be able to get away singly and therefore they get attached together to the erythrocyte.

To explain the phenomenon of multiple infection alone, it may not be necessary to presume premature rupture, because it is reasonable to expect that when there is a group of merozoites, there is every chance of two or more merozoites getting deposited on the same erythrocyte at the same time, since each individual merozoite is small compared with a corpuscle in the vicinity. It is a simultane-

ous invasion. It will be a question of random chance of deposition, between the number and size of the erythrocytes on the one hand and the number and size of the merozoites on the other, in the small space surrounding the groups of merozoites which have just burst from the schizont. The common occurrence of multiple infections in the case of *Plasmodium falciparum* may be for the reasons that 1) *Plasmodium falciparum* merozoites are the smallest (the merozoites of *Plasmodium falciparum quotidianum* are generally less than $0.5\ \mu$ in size), 2) Heavy infections of *Plasmodium falciparum* are more common, 3) Schizogony takes place in the internal organs and capillaries where there is crowding, 4) Infected cells in capillaries and internal organs have a tendency to clump together, more than in the case of *Plasmodium vivax* infections. But to explain (a) the supposed process of actual division going on in the ring stage and (b) the presence of fine filaments connecting the parasites, the hypothesis is useful.

Alassandrin had observed in fresh blood actual division going on. This division was supposed to be binary fission; but it can be explained by imagining that the division is only a continuation of the process of separation of the merozoites which should have been completed in the schizont itself but for the premature bursting. Opposed to this observation of Alassandrin is Wenyon's experience that he could not observe fission or conjugation. Craig who had believed in conjugation gave up the theory finally.

The presence of fine filaments joining the merozoites is due to the non-separation of the merozoites from one another completely. If the rupture takes place just immediately before maturity, the process of separation of the merozoites may continue even after they get deposited on the red blood corpuscle, and either the separation may be completed leaving behind no signs that the parasites were from the same schizont, or further separation may stop in the middle leaving behind cytoplasmic filaments connecting the parasites. But in case the bursting takes place considerably in advance of maturity, then the process of separation may not continue fully because the time has arrived for the process of growth to commence and therefore the process of division stops, and hence we find that even in fully grown double parasites there is often a filamental link between the two parasites.

Wenyon (6) observes that the interpretation of the phenomenon of multiple infection as an attempt at a primitive method of binary or multiple fission at a very early stage of development, is highly speculative.

All the appearances found in multiple infection can be explained on the supposition that the merozoites are not separated from one another completely when they get deposited together on erythrocytes, and in cases where no connecting strands can be seen it may be that the parasites were separated fully after deposition, or two or three individual merozoites were deposited simulatensouly. The rings may run into one another or overlap, giving rise to many appearances for which binary fission has been offered as an explanation. The hypothesis suggested in this article will very well explain all these.

The appearances in the photo-micrographs in the articles on binary fission by Hingst (1) and Beach (2) can be explained with the help of this hypothesis.

The photographs on page 23 of Thomson and Robertson (3) and on pages 404, 439 and 445 of Craig (5) are very suggestive.

This explanation for multiple infection is suggested as a result of the study of preparations examined in routine laboratory work. It is hoped that this hypothesis will create a fresh interest in the study of multiple infections.

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PACIFIC VIVAX MALARIA IN THE AMERICAN NEGRO

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It has long been thought that the negro of the Southern United States has a peculiar and characteristic tolerance to the strains of *Plasmodium vivax* to which he is commonly exposed. The scope and nature of this racial tolerance to vivax malaria, however, remains somewhat obscure.

In 1933 Boyd and Stratman-Thomas (1) published the results of experiments in which efforts were made to experimentally infect southern negroes with vivax malaria of domestic origin by means of large numbers of infected mosquitoes. Of six adult negroes thus exposed to infection in one series, three failed to develop malaria, two developed very brief or irregular fevers, and one developed a six-day fever twelve days after infection followed by one brief recurrence. One negro in this group resisted application of thirteen infected anopheles, although application of four infected anopheles invariably produced malaria in white subjects. In another experiment a group of infected mosquitoes were fed on six white patients, 2-3 mosquitoes per patient; then on two negroes, 17-18 mosquitoes per negro patient; then on an additional six white patients, 2-3 infected mosquitoes per patient. All white patients developed malaria within 12-20 days, and neither of the negroes developed infections. In a third experiment efforts were made to infect a five-year old negro child. Suitable white controls were employed, and as before the controls developed infections whereas the negro child remained well. The latter experiment would suggest the presence of natural immunity. The experiments on adult negroes clearly indicate a racial tolerance, either natural or acquired, to vivax malaria.

Bispham (2) in 1943 also called attention to the resistance of the southern negro to *P. vivax*. In a survey of Georgia school children it was found that while 7.5 per cent of negro school children showed a malaria parasitemia as compared with 1.4 per cent of white school children, only 18.6 per cent of the negro parasitemias were *P. vivax* as compared with 57.2 per cent among the white group. It was noted that in negro adults the relative decrease of *P. vivax* parasitemias was even more pronounced.

The work reported by Bispham suggests acquired rather than natural tolerance, but it must be remembered that while the southern negro and rural white have lived side by side for generations with comparable exposure to the same strains of vivax malaria, differences in racial tolerance apparently exist. Many factors, however, render conclusions difficult in regard to the nature of the negro and white response to vivax infections in the United States. Prime among these are differences in the amount of malarial therapy available to the two races, the possible African origin of the strains of *P. vivax* present in this country and the relative historical exposure of the two races to vivax malaria.

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With the onset of the present war in the Pacific and the arrival of negro troops on highly malarious bases in the South Pacific (Melanesia), it was hoped that the negro might be spared the ravages of Pacific vivax malaria because of his racial tolerance to the United States strains. It was early noted that vivax malaria of the South Pacific among white American troops was characterized by frequent, recurrent, acute attacks and a prolonged course, and was productive of great loss in time and efficiency on advance combat bases. The availability of troops possessing actual resistance to vivax, partial or complete, would naturally be a military asset of importance.

A group composed of a substantial proportion of colored troops located on a malarious island were followed for a period of eleven months (1942-1943). Statistics were collected under the stress of wartime expediency on an advanced base. Although it is unfortunate that they are not more complete, those presented are accurate due to the presence of adequate diagnostic facilities. They clearly indicate the relative effect of South Pacific *P. vivax* on the American white and negro.

TABLE I

Percentage distribution of population and malaria in study group according to race

	RELATIVE SIZE OF EACH GROUP	TOTAL MALARIA FOR MONTH	PRIMARY ADMISSIONS FOR MONTH	READMISSIONS FOR MONTH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
White.....	72	65	68.2	58.5
Negro.....	28	35	31.8	41.5

The group under observation consisted of several thousand troops. Twenty-eight per cent of the group were negroes. These negroes were almost entirely from the Carolinas and Georgia, and were in the 20-35 age group. Virtually no malaria had been noted among the group during their training period in the United States or during their voyage to the South Pacific. Considerable exposure to infected anopheles (*Anopheles farauti* Lav.) is evidenced by a sizeable incidence of initial attacks during the period of study. Suppressive atabrine, while employed was not taken regularly, as is proven by the continued incidence of initial and recurrent attacks and the subsequent absence of an increase in incidence when suppressive treatment was discontinued.

The incidence of primary admissions and readmissions for malaria was calculated monthly for each organization and racial group. Readmissions include recurrences and some reinfections as these two categories cannot be distinguished in a malarious area. Species of plasmodia among total primary admissions and readmissions were also calculated monthly, but unfortunately were not calculated for racial groups. However, this omission is not a serious handicap in forming statistical conclusions regarding racial susceptibility to vivax malaria, as will be seen.

Table I presents a percentage breakdown of the distribution of primary, readmission and total malaria among the two racial groups during a sample

month. For reasons of military security incidence rates cannot be revealed. However, the total number of malaria attacks occurring in the study group during the month in question was 160. Table II presents a percentage breakdown of the 160 malaria attacks according to species of *Plasmodium* and type of admission, i.e., primary admission or readmission.

It is noted in Table II that 90.5 per cent of all readmissions were due to *P. vivax*, and 9.5 per cent were due to other species, species undermined and mixed infections including *P. vivax*. In Table I it is noted that 41.5 per cent of all readmissions occurred in the negro group. Conceding that the entire 9.5 per cent of non-vivax readmissions occurred in the negro group, the remaining 32 per cent of total readmissions falling within the negro group must of necessity have been due to *P. vivax*, thus conclusively establishing the existence of a sizeable vivax incidence in the negro group. In this connection it is noted that the proven vivax readmission rate among the negroes is in excess of the total readmission rate for the white group.

TABLE II
Percentage distribution of species according to type of admission

SPECIES	PRIMARY ADMISSIONS	READMISSIONS	TOTAL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>P. vivax</i>	68.2	90.5	75.6
<i>P. falciparum</i>	13.1	3.8	10.0
<i>P. malariae</i>	2.8	0.0	1.9
Mixed infection (<i>falc. and vivax</i>)	1.9	1.9	1.9
Species undet.....	14.0	3.8	10.6

In considering primary admissions it is seen that 68.2 per cent of the total primary admissions were caused by *P. vivax* and 31.8 per cent were either species undetermined, other species or mixed infection including vivax. By a coincidence exactly 31.8 per cent of total primary admissions occurred among the negro group. Therefore, theoretically all the non-vivax primary admissions might have occurred in the negro group. However, one-half of the non-vivax group of 31.8 per cent was made up of mixed vivax-falciparum infections and undetermined species which were in all likelihood partly vivax cases.

A similar consideration of total malaria (primary admissions and readmissions) reveals that after making the foregoing theoretical concession for all non-vivax cases 10.6 per cent of the total vivax cases must still fall within the negro group.

Figure 1 graphically presents the relative incidence of primary and readmission malaria in the white and negro groups for an eleven months period. The relatively high incidence of readmissions among the white group during the first six months is due to the epidemic experience of a portion of this group, just prior to the arrival of negro troops. Other fluctuations in rates are attributed to the varying efficiency of suppressive atabrine administration among different groups from time to time; and varying local exposure from month to month. Except for the first three months of the period covered by the graph, vivax malaria

SUMMARY AND CONCLUSIONS

According to the literature the American negro possesses a marked tolerance, either natural or acquired, to the vivax malaria which he encounters in the United States.

A group of American white and negro troops, stationed in a highly malarious area of the Pacific, were studied to determine their comparative response to Pacific vivax malaria. There was no difference in the incidence of primary or recurrent malaria between the two races. Clinical and epidemiological observations likewise indicated a similar behavior in regard to this disease. Natives of this area in comparison with American negroes and whites manifested definite tolerance to the vivax malaria of their neighborhood.

It is concluded that:

a. The American negro lacks racial tolerance to the strain or strains of vivax malaria encountered in the Pacific.

b. The susceptibility of American negroes to Pacific vivax malaria does not differ from that of the American white.

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FUADIN THERAPY IN 150 CASES OF SCHISTOSOMIASIS MANSONI WITH A FOLLOW-UP STUDY OF 70 CASES¹

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Considerable attention has been focused on the problem of tropical disease in military personnel returning from tropical regions. The North American physician is viewing with renewed interest such conditions as malaria, filariasis, amebic dysentery, scrub typhus, leishmaniasis, and schistosomiasis. The last mentioned condition is of particular interest to us, as the writers are stationed in Puerto Rico where *Schistosoma mansoni* is endemic. It is significant to note that of the large numbers of American soldiers coming from within the territorial limits of the United States and stationed on this island for variable periods ranging in some instances to over 3 years, no case of schistosomiasis was encountered. This, in spite of the intensive military training program and manoeuvres that have been conducted in various parts of the island. In the processing of men between the ages of 18 and 38 in Puerto Rico for induction into the Army in 1944, 14.6 per cent or 2,326 of 15,831 men who had passed all their physical requirements were found positive for ova of *Schistosoma mansoni* in 1 stool examination employing the concentration technique.⁴

The 3 species of Schistosomes, each having distinct and widespread geographical distribution with some overlapping, specific intermediate hosts, produce somewhat the same general pathological picture in the human. *Schistosoma hematobium*, the cause of vesical schistosomiasis, is found in South Africa and Egypt. The only species found in the Western Hemisphere (Venezuela, Dutch Guinea, Brazil, Puerto Rico, and Lesser Antilles), although it is endemic elsewhere (Egypt and regions of South Africa), is *Schistosoma mansoni*. *Schistosoma japonicum* is endemic in China, Japan, Formosa, and the Philippine Islands, particularly Samar, Leyte, and northern Mindanao. Human beings may contract this disease by drinking of, or by wading or bathing in polluted bodies of fresh water such as rivers, streams, ponds and lakes. A specific intermediate host, a fresh water snail, must be present in the polluted waters for the transmission of the disease.

The question of the possible transmission of schistosomiasis from the returning soldiers to the civilian population in the continental United States, is an important one. The probability that certain fresh water snails may serve under

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⁴ These examinations were carried out at the Antilles Department Medical Laboratory, under the supervision of Majors Carlos A. Pons and Thomas H. Weller.

favorable conditions as the intermediate hosts must be borne in mind. Following World War I a number of Australian soldiers who had acquired *Schistosoma hematobium* infection in Egypt returned to Australia. For a time it was feared that the disease might become established in Australia in view of the discovery that certain native snails were capable of acting as the intermediary hosts of the fluke. At present it is believed that the disease is stamped out (1).

The present report discusses the use of fuadin, an antimony preparation, in the treatment of schistosomiasis mansoni. All the patients treated were native Puerto Rican soldiers who had acquired the infection on the island, probably in their childhood. Individuals affected with this blood fluke or flat worm may remain asymptomatic or present various clinical manifestations. The finding of schistosome ova in the stools of apparently healthy individuals is a relatively frequent one. Unlike the bacterial diseases, the parasites do not multiply within the human host. Thus, the clinical manifestations of the disease are dependent upon the intensity and frequency of exposure to infection. In other words, the appearance of symptoms and signs, provided the factor of immunity is constant, is in direct proportion to the number of cercariae (larval forms) which enter the body at a given time, as well as the frequency of exposure. Itching of the skin at the site of larval penetration is common. Urticaria, fever, leucocytosis and eosinophilia may follow. No case presenting these early manifestations of the disease was encountered. The cercariae after penetration of the skin, pass into the circulation, and establish themselves in the portal system where they mature. Their course of migration in the body is strictly intravascular. Mature male and female worms wander against the blood stream, and copulate in the large venous tributaries of the portal vein, showing a preference for the colonic and rectal branches. The females travel alone and reach the visceral venules into which they fit snugly and it is here that the deposition of their ova takes place mainly in the venules of the large intestine. How the ova gain access from the venules to the lumen of the colon and rectum and thus to the feces is still not clear. Bercovitz and associates (2) have recently described the presence of frequent, small, pinpoint, bleeding areas of abrasion in the rectal mucosa which suggest these sites as possible regions where the spined ova pass through the mucosa into the lumen. Material removed by swab from these bleeding points have been found to contain schistosome ova.

Many of the clinical manifestations of schistosomiasis when present, are referable to the colon, rectum, and liver. Chronic diarrhea with mucus and blood in the stool, and abdominal pain are the outstanding symptoms. In chronic and severe infections, irreversible damage is produced particularly in the liver, with resultant portal cirrhosis, congestive splenomegaly, and hepatic insufficiency. The patient may then present a clinical picture of Banti's syndrome with emaciation, hepatosplenomegaly, ascites, anemia, leucopenia, and thrombocytopenia. Rupture of esophageal varices with massive hemorrhage, hepatic insufficiency or an intercurrent infection are frequent causes of death in this late phase of the disease.

The ideal treatment in schistosomiasis is preventive. After the parasites

and their ova establish themselves in the host, the best form of therapy obviously will be unable to reverse the mechanical and inflammatory changes brought about by their presence. The present aim of therapy is that of killing the adult worms by the selective toxic action of some chemical compound, at the same time enhancing the protective powers of the host which may perhaps bring about resorption of the parasites. Treatment is advisable as soon as the diagnosis is made and every effort should be directed towards an early diagnosis. Koppisch (3) emphasizes prompt treatment in view of his portmortem observations of early involvement of the liver by the ova even in cases with minimal infection. According to this investigator, ova are constantly accumulating in the liver and producing a fibrosis which will in time give rise to an irreversible cirrhosis. Koppisch⁵ further states that fuadin has no effect on the pathological changes already established in the tissues.

The antimony compounds are the most effective preparations discovered to-date (4, 5). McDonagh is given credit for introducing intravenous antimony in the form of tartar emetic in 1915, but the treatment did not attract much attention until he and Christophenson in 1918 demonstrated its value. Potassium and sodium antimony tartrate were also widely employed. In attempting to find a less toxic antimony preparation M. Khalil Bey and M. Betache (6) working in conjunction with the chemical section of Bayer developed fuadin after a period of six years. The first report on the use of this drug appeared in 1930 and was based on the treatment of 2,041 cases of schistosomiasis in Egypt. All cases harbored *Schistosoma hematobium*, with the exception of 45 cases with *Schistosoma mansoni*. The authors felt that the results obtained in the treatment of cases with *Schistosoma hematobium* corresponded closely to those of *Schistosoma mansoni* infection. They compared the results with tartar emetic and found not only a marked reduction in the disagreeable toxic effects of tartar emetic, but a larger percentage of cures attained over a shorter period of therapy. A course of 9 injections of fuadin was sufficient to cure the majority of cases.

Fuadin is a trivalent organic antimony compound having the formula expressed as Antimony III—pyrocatechin—disulphonate of sodium (according to H. Schmidt), containing 13.5 per cent antimony. It is marketed by the Winthrop Chemical Company of New York in the form of a clear isotonic solution containing 6.3 per cent of the drug, each cubic millimeter representing 8.5 milligrams of trivalent antimony. The drug is administered in liquid form and is injected intramuscularly in the gluteal region. Pain at the site of the injection is not uncommon.

MATERIAL AND METHODS

During the past 2 years, the authors have employed fuadin in the treatment of 150 cases of schistosomiasis mansoni at the 161st General Hospital. It was the purpose of this study to evaluate under certain conditions the effectiveness of fuadin in a fairly large group of individuals. All patients were hospitalized during the period of treatment and follow-up, thus making available opportunity

⁵ Personal communication.

for close clinical and laboratory observations and study. All patients were native Puerto Rican soldiers ranging in age from 19 to 38. History and physical examination in addition to a complete blood count, Kahn test, and urinalysis, were performed on each patient. Liver function tests were performed in many cases prior to and following the administration of fuadin. Electrocardiographic tracings were also obtained. The results will be the subject of a future report. The diagnosis was made by finding ova of *Schistosoma mansoni* in the stools. In addition to a direct smear of the feces, the De Rivas (7) acid-ether technique was employed using 50 per cent hydrochloric acid instead of acetic acid. In 40 per cent of individuals treated the finding of schistosome ova in the stools was incidental to the condition for which the patient was hospitalized. The remaining patients were considered to have mild or moderately severe infections as determined by clinical criteria. The few cases presenting late stages of the disease in the form of Banti's syndrome were not treated with fuadin and are not included in this study. Fuadin was administered over a period of 17 days as follows: 1.5 cc. on the first day, 3.5 cc. on the second day, and 5 cc. on the third day and on alternate days thereafter, until a total of 10 injections was given. A course consisted of 45 cc. given in 10 injections. The amount of drug in one course was purposely limited to 45 cc. which is the quantity now recommended by the manufacturers to comprise one treatment. It was the authors' intention that this amount constitute the unit of treatment in present study.

Inasmuch as the Army induction program in the island disqualified men harbouring schistosome ova in their stools (as determined by 1 stool examination) during the period this study was in progress, the cases treated represent those men inducted prior to the establishment of this policy in early 1943 and those who had been missed by the 1 stool examination after this date. The examination of 1 stool is notoriously inadequate in ruling out schistosomiasis. In 1 individual in whom the disease was suspected on the basis of epidemiological and clinical evidence, 16 stools were examined before the first positive one was found.

The criterion used to determine the immediate results of treatment in all cases was as follows: beginning on the second day after the last fuadin injection, 5 consecutive daily stool specimens were examined for ova. If all 5 specimens were negative the patients were discharged from the hospital. In some instances where ova were still present after one course of therapy, a second course of fuadin was administered after a 10 to 14 day rest period before discharge. Although a greater number of stool specimens, over a longer period of time, would have served as a better criterion of the effectiveness of therapy, circumstances made it necessary to adopt 5 as an arbitrary number of specimens in evaluating the immediate effect of therapy and also in serving as a guide as to further fuadin therapy at the time of first hospitalization. In the follow-up study as many of the 150 individuals as could be found were recalled. Unfortunately, many of the original group were not further studied as they had departed from the island for duty elsewhere. Those available were readmitted to the hospital from 1 to 24 months following the initial course of therapy. An interval history, physical examination and a series of at least 5 stool specimens were examined. In many instances,

when 5 negative stools were obtained, additional specimens were examined. Some patients had as many as 20 negative stool examinations during the follow-up period. If the ova of *Schistosoma mansoni* were found then further fuadin therapy was instituted in some cases. Several subsequent admissions to evaluate results of therapy were obtained in these cases.

SYMPTOMS AND SIGNS PRESENT BEFORE TREATMENT

Of the 150 patients treated 55 were asymptomatic. The frequency of symptoms and signs encountered in the remaining 95 patients, is given in Table I.

TABLE I

Frequency of symptoms and signs in 95 individuals with Schistosoma mansoni ova in the stools

	Cases
Pain, abdominal, generalized.....	22
Pain, epigastric.....	22
Intermittent diarrhea, not bloody.....	19
Tenderness over descending colon.....	18
Intermittent bloody diarrhea.....	17
Weakness.....	17
Weight loss.....	16
Nausea, anorexia.....	13
Pain, left lower quadrant.....	12
Tenderness generalized abdominal.....	8
Palpable liver.....	7
Vomiting.....	6
Constipation.....	6
Hemorrhoids, external.....	4
Solid stools with blood.....	4
Pain, right lower quadrant.....	3
Tenderness over right lower quadrant.....	3
Prolapse of rectum.....	3
Palpable spleen.....	1

The evaluation of the above symptoms was difficult. In a large number of cases these appeared after entry into the service, although infection probably had taken place several years earlier. A few individuals developed symptoms after they were informed that they had schistosomiasis, while others gave conflicting histories to various medical officers or to the same officer on different admissions. A small group presented multiple vague complaints that did not fit into any one picture except perhaps an anxiety state. All individuals with a history of diarrhea with or without blood, were examined for bacillary and amebic dysentery and in few instances the results were positive. The positive cases were excluded from the analysis above. The writers are unable to explain the prolapse of the rectum in three cases as due entirely to schistosomiasis. We should like to mention that anemia was not a significant feature in the cases studied. This is surprising in view of the chronic low grade loss of blood from the rectal mucosa which may occur in schistosomiasis.

PRESENCE OF OTHER PARASITES

In areas where *S. mansoni* is endemic the presence of other parasitic infections is common. Of the 150 patients, 80 per cent harboured parasites other than *S. mansoni*. The parasites most commonly encountered were *Trichuris trichiura* in 58 per cent of the cases, hookworm in 44 per cent, and *Strongyloides stercoralis* in 12 per cent. Other intestinal helminths and protozoa were found less frequently such as *Ascaris lumbricoides*, *Endamoeba histolytica*, *E. coli*, *E. nana*, *C. mesnili*, and *Giardia lamblia*. These parasites occurred singly or in combination in the individual cases. Four patients had asymptomatic *W. bancrofti* infections and 4 others had malaria (*P. vivax*).

TOXIC MANIFESTATIONS OF FUADIN

Toxic reactions following fuadin, although mild in most instances, were recorded in approximately 20 per cent of the cases. Pain in 1 or more joints appeared in 15 per cent of those treated. The shoulders, knees and elbow joints were the most commonly involved. No evidence of local inflammation or limitation of motion was observed. Seven patients who developed joint pains received more than 1 course of fuadin. The symptoms occurred during the second course of fuadin in 5 cases and in 1 case during the second and fifth courses. The other case had joint pains during the first series of injections and had no reaction during the second series. A few patients complained of pain in the calves of both legs, a few others of anorexia, nausea and dizziness. One patient had hallucinations following the fourth injection. He was subsequently treated with 2 further courses of fuadin and had no reaction to the drug. In general the toxic reactions were mild, the complaints lasting 1 to 2 days, usually occurred late during the course of treatment and were not of sufficient intensity with few exceptions, to interrupt completion of the course. Two cases merit separate consideration.

In one case, a severe reaction characterized by sudden generalized abdominal pain, accompanied by temperature of 102°F. and a leucocyte count of 34,000 with 79 per cent eosinophiles was observed following the administration of a total of 15 cc. of fuadin. The reaction appeared 12 hours after the fourth injection, and on the fifth day of treatment, and lasted 10 days, during which time the patient complained of intermittent fever, abdominal pain, malaise and anorexia. On admission to the hospital the total leucocyte count was 10,000 with 20 per cent eosinophiles. Although the leucocyte count gradually returned to normal the percentage of eosinophiles fluctuated between 40 and 50 per cent. Treatment was resumed with doses of 2.5 cc. every other day until a full course of fuadin was given. It was suggested that this reaction represented a foreign protein reaction perhaps due to the disintegration and absorption of dead worms.

In another patient, a large painful swelling in the right buttock occurred after administration of the ninth injection. A daily temperature rising to 103°F. and a maximum leucocyte count of 19,000 with 21 per cent eosinophiles was observed. A pyogenic abscess was suspected, but incision revealed no pus. The soft tissues were edematous and indurated. The local and systemic reaction lasted 6 days.

No further fuadin was administered. On admission the leucocyte count was 8,000 with 11 per cent eosinophiles.

No other parasitic infestations were found in the 2 cases discussed.

Fuadin, although less toxic than antimony preparations formerly employed, should be used with extreme caution, if at all, in debilitated patients or in patients with myocarditis, nephritis, or hepatitis.

IMMEDIATE RESULTS OF TREATMENT

The results of the stool examinations following the termination of treatment in 150 cases is presented in Table II.

The examination of the stools immediately after completion of a course of therapy yielded extremely favorable, yet misleading results. Of the 150 patients, 114 or 76 per cent had 5 negative daily consecutive stools, following the first course of fuadin. The first of the 5 specimens was collected the day following the last injection. A second course in 21 individuals that were positive

TABLE II

Immediate results of treatment with 1 and 2 courses of fuadin in 150 cases

	CASES	NO. OF PATIENTS WITH 5 NEGATIVE STOOL SPECIMENS	PER CENT OF CASES WITH NEGATIVE STOOLS
1 course fuadin.....	150	114	76
2 courses fuadin.....	21	15	10

following the first course yielded favorable results in 15 cases. In other words, 86 per cent of the cases treated with 1 or 2 courses of fuadin did not show demonstrable ova within a week following completion of therapy. In a few individuals stools were examined daily for a period of ten to fifteen days following 1 or 2 courses of fuadin, with negative results.

FOLLOW-UP STUDY

It was possible to study an unselected group of 70 patients of the original series of 150 men over a period of 1 to 24 months following treatment. The individuals were readmitted to the hospital for observation. An interval history, physical examination were made and stools were examined daily for at least five days. History of possible reinfection following treatment was denied by all the patients.

In Table III are shown results of stool examinations in 70 individuals observed at monthly intervals following treatment. Fifty-five individuals had received 1 or 2 fuadin courses (45-90 cc.), and 15 others had been given more than this amount but not over 6 courses, a total varying from 135 to 270 cc. fuadin. More significant evaluation of therapy would have been obtained had it been possible to follow a larger group of cases which had received more than 90 cc. of the drug.

Thirty-nine cases or 55.7 per cent were found to have positive stools, while thirty-one or 44.3 per cent showed negative stools for schistosome ova.

In Table IV are shown the number of men with symptoms and those symptom-free with and without schistosome ova in the stools at the time of the follow-up

study. The entire group of 70 individuals was included in the Table irrespective of the quantity of drug administered. The most common symptoms noted on readmission were abdominal pain, bloody stools and diarrhea. Twenty-five men or 35.7 per cent had symptoms and in each case schistosome ova were found in the stools. There were 15 instances or 21.4 per cent, with symptoms but with

TABLE III

Results of stool examination in 70 individuals observed at monthly intervals following treatment

FUADIN [®] COURSES (45 cc.)	MONTHS FOLLOWING TREATMENT																				TOTAL CASES	PER CENT		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	24							
Cases with positive stools, 39																								
1	1	2	2	4	3	1	1	1	1	2	1	1	2	1									23	55.7
2		1				1		1			3		1	1	1								9	
3											1	2			1								4	
4											1												1	
5												1	1	1									2	
Cases with negative stools, 31																								
1				2		3	3	1		1	4	1	1	1									17	44.3
2						1			1	1		2	1										6	
3		1							1				2							2			6	
4																			1				1	
6													1										1	
																						70		

TABLE IV

Results of stool examinations in 70 symptomatic and asymptomatic individuals examined from 1 to 24 months following treatment with fuadin

	MONTHS FOLLOWING TREATMENT																				TOTAL CASES	PER CENT
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	24					
Symptomatic, 40 cases																						
Positive stools.....	1	2	1	3	2	2	1	1	1	1	4	2	2	1	1	0	0	25	35.7			
Negative stools.....	0	1	0	1	0	2	2	0	1	1	3	1	2	0	0	0	1	15	21.4			
Asymptomatic, 30 cases																						
Positive stools.....	0	1	1	1	1	0	0	1	0	1	2	2	2	1	1	0	0	14	20.0			
Negative stools.....	0	0	1	0	0	1	2	1	1	1	1	2	3	1	0	1	1	16	22.9			

no detectable ova in the stools. Fourteen cases or 20.0 per cent were symptom-free but showed positive stools. Sixteen other cases, 22.9 per cent, in the asymptomatic group had negative stools. These results on the relationship of symptoms to the presence or absence of ova in the stools are not considered significant. As already stated the evaluation of symptoms and complaints was difficult and conflicting statements were given by the same patients on different admissions.

COMMENT

In the English literature, few reports are available on the use of fuadin as a therapeutic agent in the treatment of *Schistosomiasis mansoni*. In spite of the introduction of fuadin in Egypt and its extensive use in *S. mansoni* and *S. hematobium* infections there is a paucity of follow-up data. During the years 1931 to 1933 inclusive, Khalil Bey (8) at the Research Institute in Cairo treated 8,889 cases. Of this number, 802 were reexamined 1 month following completion of therapy and 92 cases or 11.5 per cent were positive. It was not clear as to the number of *S. mansoni* cases in this group. Rodriguez-Molina and Juan A. Pons (9) from Puerto Rico, have employed fuadin in the treatment of *S. mansoni* for over 10 years, without untoward reactions. However, no follow-up studies were made. Hoff (10) and Ortiz (11) in 1936 have mentioned its use in a few cases. Here again there was no follow-up study. Cawston (12) working in South Africa, stated in 1937 that the vast majority of cases receiving fuadin are incompletely cured of bilharzia infection and believed this to be due to relatively low per cent of antimony (13.5 per cent) in the drug. While this present study was nearing completion, Hernandez-Morales (13) working at the School of Tropical Medicine in San Juan, Puerto Rico, reported on the use of fuadin in 157 cases of schistosomiasis mansoni. Ninety-two cases or 58.6 per cent who had been observed for a "reasonable period" of time had no ova in their stools following fuadin therapy. Sixty-three or 40.1 per cent had no ova in their stools after the first course of fuadin injections; 21 or 13.3 per cent after the second course; 5 or 3.1 per cent after the third course; and 3 or 1.9 per cent after the fourth course of fuadin treatment.

In our present study of 150 patients, one may gather the impression that fuadin is a fairly effective drug as judged by the results of stool examinations immediately following therapy. Seventy-six per cent were negative following one course of fuadin and an additional 10 per cent following a second course. It is likely that the drug may have brought about an inhibitory action on the ovipositing female schistosomes that were not killed, thereby rendering the stools free of ova for a certain period immediately after treatment.

The follow-up studies in 70 individuals presents a distinctly less favorable impression. Fifty-six per cent or 39 of these 70 men examined 1 to 24 months following treatment continued to pass ova in their stools. Seven patients in this group received more than 2 courses of fuadin but not over 6 courses.

It is clear from our results that the examination of the stools to evaluate the effectiveness of therapy should be made after an interval of 1 month or better still at periodic intervals following completion of therapy. Examinations made immediately after completion of treatment have been found misleading.

The persistence of symptoms following 1, 2, 3, or more courses of fuadin in a large number of patients makes one wonder as to the validity of complaints in many of these individuals particularly since the complaints originated after entry into the service or after the diagnosis was made as a result of a routine stool examination. For these reasons we believe that the evaluation of therapy in the

group studied should be made on objective observations rather than on an analysis of subjective complaints.

We believe that the utmost caution should prevail in the evaluation of any treatment when a criterion for such treatment is based upon the presence or absence of ova in stools, even though more reliable qualitative methods of stool examination are employed. This is particularly true in parasitic infections such as those with *Schistosoma mansoni* where the adult worms do not live in the lumen of, or on the walls of the intestine, nor are the ova deposited directly therein. Furthermore, very little is known of the *modus operandi* of fuadin *in vivo*, of its action on the mature worms living in the mesenteric veins, or upon the ova deposited by the gravid female in the venules of the colon and rectum. Nor are we familiar with the action of the drug on the oviposition of the female *Schistosomes*.

SUMMARY AND CONCLUSION

1. Fuadin was employed in the treatment of 150 Puerto Rican soldiers harboring *Schistosoma mansoni* ova in their stools. The patients studied were considered to have mild or moderately severe chronic infections.

2. No cases of schistosomiasis *mansoni* were encountered in North American troops stationed in Puerto Rico.

3. Fifty-five or 37 per cent of the individuals treated were asymptomatic prior to treatment. In 60 cases, or 40 per cent, the finding of schistosome ova in the stools was incidental to the condition for which the patient was hospitalized.

4. The frequency of various signs and symptoms encountered in ninety-five cases are presented.

5. The drug was given in courses of 45 cc. each, comprising a total of 10 intramuscular injections. The first 3 injections of 1.5, 3.5, and 5 cc. each, were given on successive days and the remaining 7 injections on alternate days. One hundred and fifty cases received 1 course of fuadin; twenty-one cases were given a second course (90 cc.) of therapy and fifteen others received 3 to 6 courses (135 to 270 cc.).

6. Toxic reactions of a mild degree occurred in 30 or 20 per cent of the cases. Two patients developed constitutional reactions.

7. The immediate effect of therapy was as follows: 114 cases or 76 per cent had negative stools after 1 course, and an additional 10 per cent following a second course.

8. Follow-up observations for a period of 1 to 24 months after treatment in 70 unselected cases revealed 39 individuals (56 per cent) with positive stools for schistosome ova and 31 (44 per cent) with no ova in the stools.

9. Fuadin, when given in 1 or 2 courses (45 or 90 cc.), is not a very efficient drug in the treatment of mild, asymptomatic, or moderately severe chronic infections with *Schistosoma mansoni*, as determined by the presence of ova in the stools.

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PARASITOLOGIC STUDY OF 400 SOLDIERS INTERNED BY THE JAPANESE

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From October 1, 1945 to March 15, 1946 four hundred American soldiers who were held as prisoners of the Japanese for more than three years were admitted to Bruns General Hospital at Santa Fe, New Mexico. A parasitologic study of these men has revealed a high incidence of parasitic infestation.

The group of men examined were all captured during the fall of Bataan and Corregidor, and were sent to Camp O'Donnell in the Philippines, many of them taking part in the historic *death march* to arrive at their destination. During the summer and fall of 1942 most of them were interned at Cabanatuan. Sometime during the next year or two they were transferred to the Japanese homeland. Here they were separated into small camps, but most remained on the island of Honshu; a few men were sent to Formosa and another small group to Manchuria.

Upon liberation, almost all the men in this group suffered from malnutrition and deficiency states, but by the time of their arrival at Bruns Hospital practically all had recovered from their malnutrition, and very few showed residual clinical deficiency states.

This summary of the parasitologic study is a report of the laboratory findings only, and no attention has been given to the clinical aspect of the cases or result of treatment for parasites.

As a routine procedure the laboratory employed the zinc sulphate flotation method of concentration. The levitated material was looped off into iodine so that both cysts and ova could be searched for in the same examination. In a few instances cultures for *Endamoeba histolytica* were done, and an occasional iron hematoxylin preparation was made. On a few specimens an additional examination for schistosome ova was made by employing an acid-ether concentration.

In this study, 1,692 stool specimens were examined, with an average of 4.2 specimens per case. No effort was made to show the relative advantage of repeated stool studies, since the advantage is already well known. It is true, however, that the presence of additional parasites was demonstrated in the third, and in a few instances, even the fourth specimen. In all cases where the patient was in residence for a long enough period it was the aim to examine at least two cold specimens and one warm liquid specimen obtained after the administration of one ounce of fifty per cent magnesium sulphate.

The study revealed the presence of parasites in 343 patients or 86 per cent. Seventy-six per cent of the patients harbored parasites that are generally considered to be pathogenic. In this group were included the nematodes *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm and *Trichostrongylus*, the cestode *Hymenolepis nana*, the trematode *Schistosoma japonicum*, the flagellate *Giardia lamblia* and the amoeba *Endamoeba histolytica*.

Table 1 shows the number of cases in which each parasite was found and the relative frequency of each.

A large proportion of the patients harbored more than one helminth. A multiple infection with *Ascaris*, *Trichuris* and Hookworm was a frequent finding. Table 2 shows the frequency of single and multiple helminth infestation.

TABLE 1

Number of cases with parasites and relative frequency of each parasite

PARASITE	CASES	PER CENT
<i>Endamoeba coli</i>	127	32.0
<i>Endolimax nana</i>	61	15.0
<i>Iodamoeba butchlii</i>	11	2.7
<i>Endamoeba histolytica</i>	45	11.0
<i>Trichomonas hominis</i>	9	2.2
<i>Chilomastix mesnili</i>	8	2.0
<i>Giardia lamblia</i>	33	8.2
<i>Strongyloides stercoralis</i>	13	3.2
<i>Ascaris lumbricoides</i>	141	35.0
<i>Trichuris trichiura</i>	159	40.0
Hookworm:.....	139	35.0
<i>Trichostrongylus</i>	5	1.2
<i>Hymenolepis nana</i>	10	2.5
<i>Schistosoma japonicum</i>	3	0.75
Total number patients.....	400	
Number infested.....	343	86.0
Number with pathogenic parasites ..	304	76.0

TABLE 2

Frequency of single and multiple helminth infestation

NO. OF WORM SPECIES PRESENT	CASES	PER CENT
1	149	37.0
2	98	24.5
3	37	9.2
4	4	1.0

Attention is called to the finding of the ova of *Trichostrongylus* in five patients. Because of the similarity of this egg to that of Hookworm and *Heterodera radicola*, erroneous diagnoses have sometimes been made by the casual observer. Ova of *Heterodera radicola* were found in thirteen patients, but since this is not a true parasite of man this figure was not included in table 1.

SUMMARY

1. Parasitologic findings on 400 soldiers are presented. These men were prisoners of the Japanese and interned in the Philippines and Japan.

2. Eighty-six per cent were found to harbor intestinal parasites, while 76 per cent harbored parasites generally considered to be pathogenic.

AGGLUTINATION OF ENDAMOEBA HISTOLYTICA CYSTS¹

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INTRODUCTION

Zaubitzer (1), Coca (2), von Schuckmann (3) and others have used the agglutination reaction in the study of free-living amoebae. Sellards (4) injected rabbits with the cysts of an unclassified species of amoeba found in lake water, but was unable to produce agglutinins for this organism. Heathman (5) noted spontaneous agglutination of free-living amoebae in normal rabbit serum. The present investigation began as an attempt to demonstrate agglutinins in the sera of carriers of *Endamoeba histolytica* for cultured cysts of this parasite.

PROCEDURES

The NRS strain of *E. histolytica* was grown on coagulated egg medium (6) in test tubes, using a modified Locke's solution overlay (NaCl 8.0 gm., CaCl₂ 0.2 gm., KCl 0.2 gm., MgCl₂ 0.01 gm., KH₂PO₄ 0.3 gm., distilled water 1000 ml. (7). Cysts were obtained by transferring the sediment from three 48 hour culture tubes to one tube containing sterile rice starch (8). After 72 hours, the contents of the starch tubes were removed, shaken with normal saline solution, and centrifuged at 1500 rpm. for five minutes, the supernatant fluid drawn off and discarded. This washing process was repeated two more times. The remaining sediment was thoroughly shaken in a solution of zinc sulfate of specific gravity 1.13 and following centrifugation, the supernatant layer containing cysts and egg particles was pipetted off. Three additional saline washings of the cyst-containing material gave a final suspension free of starch, which could be sterilized, if necessary, by washing for 45 minutes with a 1:50,000 bichloride of mercury solution. Addition of bichloride of mercury, however, necessitated three further washings with sterile saline (9).

Fresh sera were inactivated at 56°C. for 30 minutes, then kept at 4°C. until immediately before use, when serial normal saline dilutions were made. Sera previously used for routine complement fixation tests at the National Institute of Health were preserved with merthiolate and stored at 4°C. for several months prior to use. These were sera from persons suspected of being infected with *E. histolytica*. The following biological products were also tested: serum albumin (human) concentrated (Lilly Lot #352336C), dried reconstituted normal human plasma (Ben Venue Lot #2971), normal horse serum (Mulford Lot #42039A2), normal serum gamma globulin antibodies concentrated fraction II in saline (Antitoxin and Vaccine Laboratory, Mass. Dept. of Pub. Health Lot #11GS74B2).

¹ The opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

Agglutination tests were performed as follows: 0.02 ml. of cyst suspension (thoroughly shaken) and 0.02 ml. of serum were mixed on a well slide. The slide was then placed in a Petri dish containing moist filter paper and incubated at 37°C. for $\frac{1}{2}$ -2 hours with gentle agitation every 15 minutes. The slide was

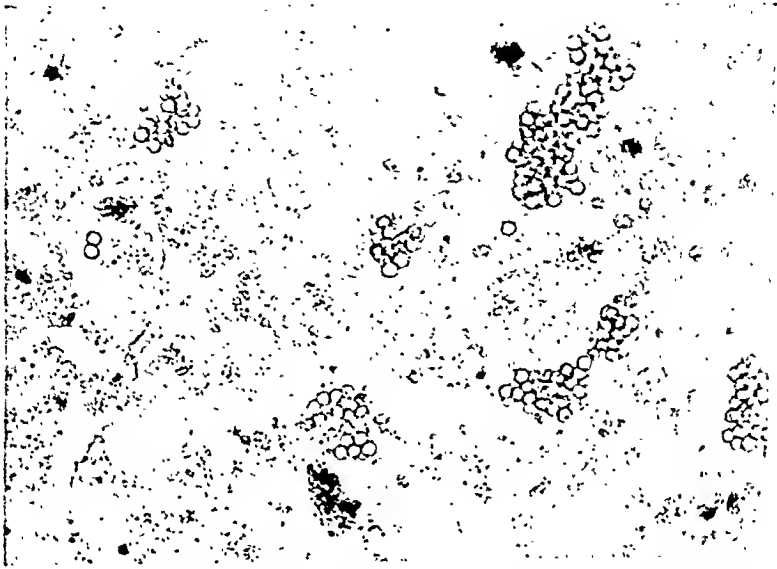


FIG. 1. PHOTOMICROGRAPH ILLUSTRATING AGGLUTINATION OF ENDAMOEBEA HISTOLYTICA CYSTS IN STRONGLY POSITIVE REACTION

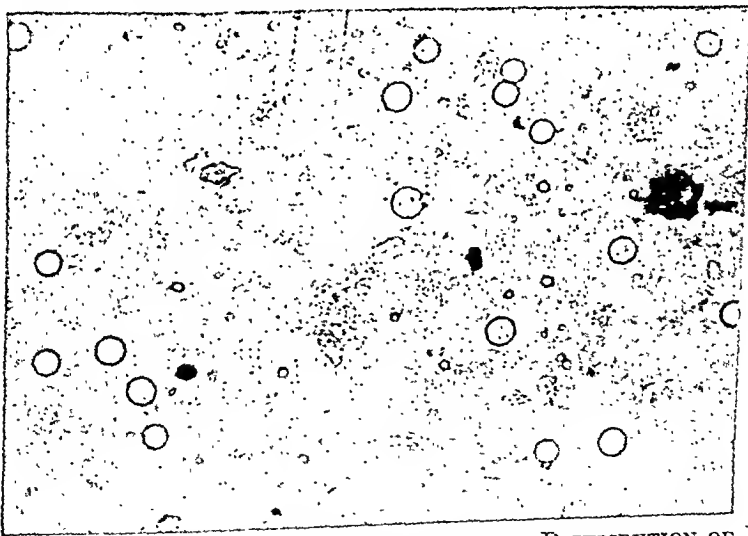


FIG. 2. PHOTOMICROGRAPH (HIGHER POWER) ILLUSTRATING DISTRIBUTION OF ENDAMOEBEA HISTOLYTICA CYSTS IN CONTROL OR NEGATIVE REACTION

shaken vigorously before each microscopic examination. A saline control and a serum known to agglutinate cysts were included with each group of unknown sera tested. Agglutination, when present, was marked at the end of two hours, but proceeded still further when the slide was kept at 4°C. for eight to ten hours thereafter. More rapid results were obtained when a Kline rotating type automatic shaker was used.

The agglutinations were recorded as + + + + when there were large clumps and very few single cysts visible (fig. 1). The smallest amount of agglutination, recorded as \pm or +, indicated many single cysts and only rare clumps of four or more organisms. Cysts in pairs or groups of three were not considered to be evidence of agglutination.

To determine whether the agglutination was a bacterial adsorption phenomenon, a 1:4 serum dilution was incubated for 12 hours at 37°C. with each of two

TABLE 1

Agglutination of Endamoeba histolytica cysts by whole sera and serum fractions

SERUM NO.	COMP. YDX.	CYSTS IN TUBES	SERUM DILUTIONS—READ AFTER 2 HRS. AT 37°C.							SERUM OVER 2 WKS. OLD	REMARKS
			1:4	1:8	1:16	1:32	1:64	1:128	1:256		
1	—	—	++++	++++	++++	++++	++	++	—		
2		+	++++	++++	++++	++++	++++	++	—		
3		+	++++	++++	++++	++++	++	\pm	—		
4	—		++++	++	\pm	—	—	—	—	+	
5	+		++++	++++	++	—	—	—	—	+	
6	—		++++	++++	+	—	—	—	—	+	
7	—		++++	++++	+++	\pm	\pm	—	—	+	
8	—		++	+	—	—	—	—	—	+	
9	+		++	++	+++	+++	—	—	—	+	
10			+++	—	—	—	—	—	—		
11			—	—	—	—	—	—	\pm		
11			+++	+++	+	—	—	—	—	+	21 days later
12			++++	++++	++++	++++	+++	\pm	—	+	Saline pH
12			++++	++++	++++	++++	+++	\pm	—	+	5.9
12			++++	++++	++++	++++	+++	\pm	—	+	pH 6.9
12			++	*	++	*	*	*	*	+	pH 7.2
Adsorb.			++	++	—	—	—	—	—		Ster. Cysts
Unads.			++	++	++	\pm	—	—	—		
Plasma			++++	++++	+++	+	—	—	—	+	
Albumin			—	—	—	—	—	—	—	+	Dil. 1:20
Gamma Glob.			—	—	+	\pm	++	+	—	+	Conc.

* Suspension of sterile cysts contained too few cysts for satisfactory tests in all dilutions.

heavy suspensions of organisms cultured from cyst tubes and killed by heat and formalization.

RESULTS

The significant data are summarized in table 1. These results show that certain fresh human sera contain agglutinins for the cysts of *E. histolytica*. From the experiments on human serum products, there is an indication that these agglutinins are contained in the gamma globulin fraction, since saline dilutions up to 1:400 of the concentrated fraction clump the cysts if the slides are held at 4°C. for eight hours following the original incubation. The agglutinins are not affected by variation of the pH of the saline diluent between the ranges

of 5.9-7.2, and are not completely removed by serum adsorption by bacteria with which the cysts were cultured. To determine the effect of holding unsterile sera at 4°C., one serum was retested 21 days after the initial results, and in this case a definite increase in agglutinating power occurred following storage. Sterile cysts, when present in sufficient numbers, were agglutinated in the same manner as cysts contaminated with bacteria.

It is of interest to note that in addition to the above, 29 fresh sera picked at random from a clinical laboratory, tested in dilutions up to 1:256, gave an occasional plus reaction in the first tube only, the remainder of the dilutions yielding equivocal or negative results. Sterile horse serum, however, contained agglutinins for cysts in dilutions up to 1:64.

SUMMARY

Agglutination of the cysts of *E. histolytica* obtained from culture occurs in dilutions of the blood serum of man and of the horse. Judging from the few cases studied, there appears to be no correlation of this agglutination phenomenon with either infection with *E. histolytica* or complement fixing properties of a serum. Further investigation of the problem seems indicated.

ACKNOWLEDGMENTS

The author is indebted to Lieutenant (jg) Leo Cravitz, H(S), USNR, for performing the bacterial adsorptions, to J. Bozicevich of the National Institute of Health, Bethesda, Maryland, for the complement fixation results, as well as for advice and assistance, to Dr. Don R. Mathieson for many helpful suggestions, and to M. R. Orlan, PhM3c, USNR, for technical assistance.

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PRELIMINARY REPORT ON FIELD EXPERIMENTS TO DEMONSTRATE EFFECTIVENESS OF VARIOUS METHODS OF MALARIA CONTROL*

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ASSISTED IN FIELD AND LABORATORY OPERATIONS BY KENNETH G. BARNHILL², AND
MICHAEL TAKOS³

PLAN OF INVESTIGATION AND ORIGINAL STUDIES

a. Five towns at sea level on the Atlantic seaboard in the Tropical American zone were chosen as the site of this experiment. The towns are situated in a line on the edge of the ocean. The towns most distant from each other are separated by fifteen miles. The remaining three towns lie in between. Meteorological factors were, for the purpose of this experiment, the same.

b. The experiment was planned to demonstrate over an indefinite long range period the effect produced by various control procedures on malaria incidence and intensity. One town was chosen to serve as a comparison area where no control was performed. Over the nine months period prior to the beginning of the experiment, surveys, including blood and splenic examinations, were performed during 1945 at approximately three to four month intervals. Dry and wet season conditions were covered by these studies. (Surveys made April, August, and December, 1945, and again in April and May, 1946.) (May survey performed only in town of "P".)

c. The original surveys in four towns performed in April 1945 showed that the malaria incidence was highly comparable in the towns selected. The splenic examinations performed on children up to 16 years of age ranged between the narrow limits of 86.3 per cent to 92.5 per cent positive, and parasite positives, with persons examined irrespective of age, between 31.1 to 44.2 per cent (dry season condition, low transmission).

d. Surveys repeated in August (wet season conditions) and early December (end of wet season conditions) showed that the splenic examinations remained approximately the same in all localities, whereas blood positives increased progressively through the wet season to reach, at the termination of the season a range of between 67.7 per cent and 92.1 per cent positive in the different towns.

e. Table 1 shows the results of splenic and blood examinations in all surveys, all towns, with date on which performed. The incidence character and size of the spleen with associated parasite positive cases and absence of any severe clinical manifestations in practically all cases examined over this period of observation, indicate that the areas can be classed as hyperendemic.

* Terminating date of this first report 3 April 1946.

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² Captain, M.C., A.U.S.

³ Technical Sergeant, M.C., A.U.S.

f. The survey of December 1945 ended the pre-study, covering dry and wet season conditions. Control measures were accordingly begun, leaving one town, as above noted, as comparison where no control procedures would be applied.

g. Dates during 1946 have been selected for the repetition of splenic and blood surveys. These dates are comparable to the period in which surveys were originally performed in 1945. They will serve as one measuring rod of results produced. Furthermore; as different methods of control are to be employed, and all the towns are highly comparable in their original incidence of malaria, it is considered that comparison between towns where differing control is being practiced, will offer an additional check on the evaluation of procedures used. It is not contemplated that all procedures will be equally effective or ineffective. The result shown in the comparison town, of course, serves, as the principal means of evaluation of procedures used.

h. The towns chosen are designated by the initials "P", "N.C.", "L", "S", and "R.I.". The last mentioned town "R.I.", was not surveyed in April,

TABLE 2

Meteorological data at seaport town on same coastline 15 miles from nearest experimental town and 50 miles from the most distant

	1945											
	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Monthly mean air temperatures....	79.8	80.2	80.2	81.0	80.5	81.8	80.4	80.1	81.1	79.4	79.2	79.8
Monthly mean relative humidity...	74.7	73.8	76.2	78.5	81.4	81.7	84.7	85.3	82.6	83.8	85.4	81.5
Monthly mean wind velocity (miles per hour).....	14.1	16.5	15.1	12.9	7.8	7.7	7.5	6.3	6.1	6.5	7.5	11.5
Rainfall inches.....	1.89	0.87	0.43	1.90	38.15	6.62	19.81	17.66	11.58	18.33	24.29	20.59

Total rainfall—132.12; excess 1945 above average—170; number of rainy days—239.

August, nor December 1945, but was first surveyed in February 1946, and again in April 1946. Originally "N.C." was chosen as comparison town, but "R.I." was later found, February, 1946, to have a comparable degree of malaria and in the same area, so it was chosen in 1946 as the control town or comparison area.

i. Meteorological observations made, at a station no more than thirty miles distant from the most distant town, are shown in table 2.

j. Original choice of control procedures in towns:

"P"—Control by means of weekly dosage of SN 7618 on basis 0.3 gm. per week for adult dosage.

"L"—Painting or spraying of interior of houses with 5 per cent DDT in kerosene. Approximate average of 2.0 gals. per house based on 300–400 mgs. per square foot.

"S"—Spraying by airplane of 5 per cent DDT in Diesel oil for a one mile radius around town, at intervals according to schedule, at rate of 0.22 lbs. per acre.

"N.C."—Comparison town. No control until April 1946, when treatment by drug 8137 was instituted after the April 1946 survey.

"R.I."—Comparison town after April 1st 1946.

k. All control procedures were begun in December 1945 following the survey of December 3rd.

l. All splenic examinations, with two exceptions were performed by Colonel John E. Elmendorf, Jr., Commandant, Army School of Malariaology.⁴

m. Blood examinations (all thick smear examinations) were performed by T/Sgt. Michael J. Takos, technician of the Malaria Parasitological Division. Thick smears were stained with Giemsa stain and considered as negative after five minutes examination.⁵

n. The town of "N.C.", chosen for comparison purposes, where no control measure was performed, is situated across the River "L", on the opposite side from town "L". The two towns lie close together being separated by approximately one half a mile. As town "L" was treated with painting of interior and, at times, exterior of houses with 5 per cent DDT in kerosene, it is believed that this procedure will produce little effect on the incidence of malaria in the comparison town "N.C.". Density of the vector in "N.C." could theoretically be influenced by painting houses in "L" with DDT as it lies within flight range distance.

DETAIL OF INVESTIGATION BY TOWNS

a. Studies in the town of "N.C." selected as comparison area.

(1) Table 3 below shows the results of the four blood and spleen surveys performed in the comparison town of "N.C." from April 1945 to April 1946.

(2) The incidence of malaria as measured by blood and spleen examinations, remained the same, the April 1945 survey corresponding almost exactly with that of April, 1946.

b. Studies in the town of "S" spraying of DDT solution by airplane.

(1) Town "S" is situated in between town "L" (painting of interior of houses with DDT) and town "R.I." (new "comparison town"), lying approximately four miles from "L", and five miles from "R.I.". Because of its surrounding uninhabited territory of a diameter of about nine miles "S" was chosen for application by airplane of 5 per cent DDT in Diesel oil. Given its relative isolation it was believed that the incidence of malaria in the other towns would not be affected directly by airplane applications.

(2) As the town is situated on an approximately straight coastline, the area for spraying consisted of a semi-circle, with a radius of one mile, measured from the edges of the town. All houses of the town proper, some forty in number, lie within one-quarter of a mile of each other.

(3) The accompanying table (table 4) shows the dates of the various spray applications with relevant associated data.

⁴ Examinations in "P" and "N.C." in December were performed by Major J. R. Gustafson, M.C., Army School of Malariaology.

⁵ The bloods from the April survey in all towns were examined by the technician of Gorgas Memorial Laboratory, under the direction of Dr. Herbert Clark. The ability of both these technicians of the Army School of Malariaology and of Gorgas Memorial Laboratory is considered to be comparable and of high calibre.

(4) The total period covered by the spraying recorded above corresponds to the end of the rainy season and practically all the dry season. By the application of 5632 pounds of DDT to this area during the dry season, it was believed that a substantial reduction could be made in the density of the vector during the breeding season, May to December 1946. The splenic and blood surveys performed in "S" are recorded in table 5.

TABLE 3

Splenic and blood findings in surveys performed by the Army School of Malariaology on the dates recorded, 1945 and 1946, in the town "N.C."

YEAR	TOTAL SPLEENS EXAMINED			TOTAL POSITIVE			PER CENT POSITIVE			TOTAL BLOODS EXAMINED			TOTAL BLOODS POSITIVE			PER CENT BLOODS POSITIVE		
	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.
1945	44	47	57	38	44	53	86.3	93.6	92.9	74	55	68	31	43	56	41.8	78.2	82.3
1946	30			28			93.3			56			23			41.0		

TABLE 4

Town "S"

DATE OF SPRAY APPLICATIONS	NUMBER OF LOADS APPLIED	APPROXIMATE GALLONS OF OIL	APPROXIMATE POUNDS OF DDT	APPROXIMATE POUNDS PER ACRE
27 December 1945	2	1600	704	0.21
3 January 1946	2	1600	704	0.21
11 January 1946	2	1600	704	0.21
13 February 1946	2	1600	704	0.21
8 March 1946	2	1600	704	0.21
20 March 1946	2	1600	704	0.21
28 March 1946	2	1600	704	0.21
2 April 1946	2	1600	704	0.21
Total.....		12800	5632	

TABLE 5

Town "S"

YEAR	TOTAL SPLEENS EXAMINED			TOTAL SPLEENS POSITIVE			PER CENT SPLEENS POSITIVE			TOTAL BLOODS EXAMINED			TOTAL BLOODS POSITIVE			PER CENT BLOODS POSITIVE		
	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.
1945	48	57	43	44	50	39	91.6	87.9	90.7	74	84	62	23	46	53	31.1	54.7	85.4
1946	30			26			86.6			57			35			61.4		

(5) From the data presented in the above table it is evident that after application of 5632 pounds of DDT over a four month period the incidence of malaria, as measured by the presence of splenomegaly, and incidence of parasite positive cases had not decreased when compared with the findings in April 1945. In fact, the parasite positive incidence had increased from 31.1 per cent to 61.4 in 1946. The results of the survey in the comparison town "N.C." indicate the normal incidence of malaria has remained the same in the area.

(6) As the latter part of December to the latter part of April or early May corresponds to the dry season and the period of corresponding curtailed anopheline breeding, very little new infection would be anticipated during the period. Accordingly it is hardly to be expected that application of DDT by air during the period of accepted non-transmission would influence materially parasitaemia of persons already infected. The increase of incidence of parasitaemia April 1946 over April 1945 remains unexplained.

(7) The real test of the procedure as a long range control measure will become evident when the new transmission season (May–December) is in progress and will be measured by the August and December surveys (1946).

c. Investigation in the town of "L" spraying interior of houses with DDT.

(1) The town of "L", treated December 3rd 1945 by means of spraying of houses with 5 per cent in kerosene at rate of 300–400 milligrams per square foot, showed the accompanying comparative seasonal findings of splenomegaly and parasitaemia. (See Table 6.)

TABLE 6
Town "L"

YEAR	TOTAL SPLEENS EXAMINED			TOTAL SPLEENS POSITIVE			PER CENT SPLEENS POSITIVE			TOTAL BLOODS EXAMINED			TOTAL BLOODS POSITIVE			PER CENT BLOODS POSITIVE		
	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.	Apr.	Aug.	Dec.
1945	40	95	52	37	89	49	92.5	93.7	94.2	86	120	51	38	79	47	44.2	65.8	92.1
1946	31			23			74.2			66			24			36.3		

(2) As is seen in the table of incidence of splenomegaly and parasitaemia, there has been a slight decrease in both as compared to the April 1945 survey. Here again the test of the procedure will become evident in the survey of August after the transmission season has been in progress for approximately three months.

(3) Special Investigation Procedure in town of "L"

(a) In the town of "L", where houses were painted or sprayed with DDT solution, boxes hinged to hang open on the wall were sprayed with 5 per cent DDT in kerosene at the time of the original spraying of the buildings, December 3rd. One sprayed box, brought to the laboratory 36 hours after spraying, and closed to contain mosquitoes, was immediately tested in order to determine the lethal effect on confinement inside of *A. aegypti* and *A. albimanus* mosquitoes. A box sprayed with kerosene, no DDT added, and a box with nothing applied were tested as controls for the box originally painted with 5 per cent DDT in kerosene.

(b) On December 3rd, four of the boxes, sprayed with 5 per cent DDT in kerosene were hung on the outside of a house and nine boxes suspended inside the same house for "weathering". These boxes were removed, at intervals of two weeks, and tested for their lethal capacity against the test mosquitoes. At each time these boxes were tested, the control boxes were also tested.

(c) The boxes, approximately 8" x 8" x 8", inside measurement, were constructed with a square opening in each end, 2" x 2". The opening in one end

was covered with mosquito screen wire, and at the opposite end a removable glass window was placed through which mosquitoes could be introduced inside the boxes. With a light placed at the end of the box near the screen opening, the mortality of the mosquitoes could be observed by looking through the glass covered opening toward the light. Twenty-five (25) *A. albimanus* mosquitoes and twenty-five (25) *A. aegypti* were used in each test.

(d) The lethal effect on the test mosquitoes, after confinement in these boxes, is recorded in the accompanying table (table 7).

(e) The above table is a condensation of original data as routine tests were made at two weekly intervals. After the 45th day, following original spraying, there were no more boxes "weathered" outside, available to be tested. After the March 28th test (115 days) the last inside box was used, and thereafter, the same box was tested after hanging in the insectary in the basement of the Army School of Malariology building.

(f) (The results of box tests here recorded represent a true sample of actual results. Limit of space prevents presentation of complete data.)

(4) Conclusions drawn from the facts presented in the table above:

(a) A pronounced lethal capacity as compared with comparison boxes is apparent in the first box tested $1\frac{1}{2}$ days after spraying with the 5 per cent DDT in kerosene. Obviously there was no appreciable "weathering" of this box.

(b) In the case of all inside boxes tested 16 to 129 days after spraying had been performed, the lethal capacity of these was marked and comparable.

(c) The table also shows a highly comparable duration of lethal capacity of boxes up to 45 days after original treatment with 5 per cent DDT in kerosene whether hung or weathered "inside" or "outside". (As it was not contemplated under the meteorological conditions present that boxes would retain a high degree of lethal capacity for such long periods, sufficient numbers were not prepared for weathering both "inside" and "outside" to permit the determining, at two weekly intervals, of the exact end point of such capacity after "weathering".)

(d) It is evident that weathering for 129 days did not exhaust the lethal capacity of the boxes weathered "inside" and in which mosquitoes remained five hours.

(e) The low degree of mortality in the control or comparison boxes supports the fact that residual DDT is the cause of mortality in the boxes treated with that agent. The fact that mortality exists in the comparison boxes though a low rate, once boxes have been weathered (one untreated and one treated with kerosene only), is believed largely due to the necessary handling of mosquitoes when being introduced into the boxes. This low mortality would of course maintain for all tests as all mosquitoes were handled in the same manner.

(f) The existence and persistence of killing capacity as demonstrated in the boxes does not indicate that comparable result would maintain against *A. albimanus* in the houses treated. Under the condition of the test, the mosquitoes in the boxes were forced to rest on the inner surface of the boxes, whereas *A. albimanus*, in nature, a species only slightly domesticated, might not rest, when in search of a blood meal, on the walls of DDT treated buildings sufficient time to acquire a lethal dose of DDT, when in its dry condition.

d. Investigation in town "P" which was treated with drug SN 7618.

(1) In town "P", treatment was inaugurated December 19th on basis of weekly dosage with SN 7618, 0.3 grams being administered to adults and graded

TABLE 7

Results of tests showing per cent mortality, and subsequent box intervals, of mosquitoes of *A. albopictus*, *A. triseriatus* and *A. taeniorhynchus* treated separately 3 December 1945 with 5 per cent DDT in kerosene.

Time elapsed with box and mosquitoes	Per cent mortality	Per cent mortality	Per cent mortality	Per cent mortality	Box interval in hours
Days	Per cent mortality	Per cent mortality	Per cent mortality	Per cent mortality	Days
1	2	12	85		14
2	2	34	95		14
3	2	34	100		14
5		34	100		14
1	0.0	0.0	0.0	12	16
2	8	0.0	56.0	25	16
3	8	0.0	74.0	34	16
5	14	0.0	100.0	100	16
1	0.0	0.0	0.0	0.0	45
2	0.0	0.0	0.0	0.0	45
3	0.0	0.0	44.0	96.0	45
5	0.0	0.0	100.0	100.0	45
1	6.0	6.0	0.0		72
2	10.0	8.0	10.0		72
3	14.0	12.0	48.0		72
5	20.0	24.0	96.0		72
1	0.0	4.0	0.0		115
2	0.0	4.0	0.0		115
3	0.0	8.0	8.0		115
5	4.0	12.0	98.0		115
1	12.0	0.0	0.0		129
2	12.0	0.0	42.0		129
3	20.0	0.0	54.0		129
5	32.0	0.0	100.0		129

* Box was not hung on outside nor inside. It was brought back to laboratory after spraying and tested within 43 hours.

dosage for children between the ages of five to fifteen years.⁶ No routine treatments were given to children less than five years of age.

⁶ $\frac{1}{2}$ tablet (or 0.15 grams) given the children of 5-14 years inclusive and $\frac{1}{4}$ tablet (0.075 grams) to selected children of approximately 4 years of age; not given to any children under 4 years of age.

(2) A roster of names of all persons residing in "P" was compiled on mimeographed forms. Weekly treatments with pertinent observations were noted thereon. Two officers, a medical officer and assistant, were always present on Wednesday of each week to administer the treatments personally and to note untoward symptoms. The pill was placed in the mouth, a drink of water taken and the mouth opened subsequently for inspection. Special arrangements were made with a nearby Army hospital to send an ambulance to transport anyone complaining of sickness after medication to the hospital for treatment. That this service was never utilized attests the fact that any symptoms encountered were of mild nature.

Notes relative to complaints of symptoms were recorded as follows:

Total number of individual treatments given.....	1732
Total number of minor complaints recorded.....	4

Splenic and blood findings in surveys performed by the army school of malariology on rates recorded 1945 and 1946

Total cases examined in blood survey 6/22/46 after treatment program had been offered for 22 weeks*.....	255
Total cases positive.....	15
Per cent cases positive.....	5.8
(a) Cases of above group never treated but examined.....	32
Cases positive (see footnote).....	11
Cases negative.....	21
Per cent cases positive.....	34.3
(b) Cases treated of above group of 255 examined.....	223
Cases positive.....	4†
Cases negative.....	219
Per cent cases positive.....	1.7

* Very few of the people availed themselves of the full course of weekly treatments.

† Two persons resulting parasite positive had moved from the town and had received their last suppressive treatments 21 and 49 days prior to their May 22nd positive blood findings. One case had received only one treatment two weeks prior to May Survey and one case had parasites in spite of 11 treatments.

Comparing the detail of blood findings prior to and after treatments

42 Cases <i>P. falciparum</i> , survey December 1945, negative parasites.	22 May 1946
*11 Cases <i>P. malariac</i> , survey December 1945, negative parasites...	22 May 1946
†12 Cases <i>P. vivax</i> , survey December 1945, negative parasites.....	22 May 1946
1 Case <i>P. falciparum</i> } survey December 1945, negative para-	
<i>P. vivax</i> } sites.....	22 May 1946
2 Cases of <i>P. falciparum</i> } survey December 1945, negative para-	
<i>P. malariae</i> } sites.....	22 May 1946

68

* One case of *P. malariae* in December 1945 survey was positive only to *P. falciparum* in April 3rd survey after 14 weekly treatments but was negative to all parasites May 22nd after 4 additional treatments.

† One case negative in December was positive to *P. vivax* in April after 12 treatments but was negative in May after 2 additional treatments.

Footnote: (See (a) above)

Untreated cases examined in relation to treatment period

Positive in December Survey still positive April or May.....	7-30.4 per cent
Negative in December, positive April or May...	2- 8.7 per cent
Not examined December but positive April or May.....	2- 8.7 per cent
Total cases Positive.....	11-47.8 per cent 47.8 per cent
Positive in December and Negative April or May.....	5-21.7 per cent
Negative in December and Negative April or May.....	7-30.4 per cent 52.1 per cent
<hr/>	
Total cases Negative.....	12
Total cases.....	23 99.9 per cent 99.9 per cent

SUMMARY

a. In town of "S" after application by airplane of 5632 pounds of DDT in Diesel oil over a four month's period, there was no evidence of a lowered incidence of malaria among the residents as measured by splenic or blood positive cases. In fact, incidence of parasite positives, April 1946, increased practically 100 per cent over the incidence of April 1945 and was considerably higher than the comparison town of "N.C."

b. In town of "L", the boxes sprayed with 5 per cent DDT in kerosene and after "weathering" gave evidence of a lethal capacity for mosquitoes introduced into them and forced to rest on the sprayed walls. This lethal capacity reached 100 per cent mortality for mosquitoes confined in the boxes for five hours 129 days after having been sprayed. (The evidence of malaria, however, as measured by splenic and blood positives shows practically no alteration when compared with the comparison town or compared with the survey of 1945 performed in the same town.)

c. The town of "P" has shown a marked drop in malaria incidence as measured by blood findings both when compared with the comparison town and compared with its own survey performed in April last year.

d. The comparison town where no control was performed showed blood and splenic positive cases practically the same in April 1946 as in April 1945.

CONCLUSIONS

a. Under the condition and procedure of the experiment, discussed in the body of the text, and with *A. albimanus* the accepted vector, reduction of percentage of cases harboring the parasite (human seed bed of infection) was not realized by the airplane spraying activities in "S", nor the painting of houses with DDT performed in "L" with control being practiced over a period of approximately four months. (No change in percentage of cases functioning as human reservoirs of infection.)

b. Treatment with drug SN 7618 once a week on the basis of 0.3 grams per adult was followed by a marked curtailment of parasite positive cases after

treatments. The reduction of incidence of parasite positive cases was realized with a minimum of untoward clinical manifestations following the administration of the drug.

c. The parasite reservoir of the malaria cycle necessary for immediate transmission, was greatly curtailed, temporarily at least, following weekly treatments. The persons so treated were not limited to a specifically treated geographic area to realize personal suppression of symptoms nor to effect the elimination of an ambulatory reservoir of parasites.

RADICAL CURE OF AVIAN MALARIA (*PLASMODIUM CATHEMERIUM*) WITH SN 8557, A NAPHTHOQUINONE DERIVATIVE¹

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In a search for curative agents for malaria a few compounds previously tested therapeutically and prophylactically were used in a variety of ways to devise routine tests for curative effect on *Plasmodium cathemerium* in the canary. The diverse behavior of sporozoite induced and blood induced malaria as well as the strain differences within a given species of malaria in both man and birds were considered in setting up the tests described here. A further consideration was the development of a short term test as compared with the more proper long term test.

The strains of *Plasmodium cathemerium* used in these experiments are 3H2-1 (derived from 3H2 by repeated blood transfers, Gingrich and Schoch, 1946) and 3C (Huff, Boyd and Manwell, 1944). Intravenous inoculation of 0.5×10^6 blood parasites of the former strain produces a relatively mild infection with a fatality rate of less than 1 per cent and no exoerythrocytic forms. The same strain induced by intravenous inoculation of sporozoites (equivalent of 1 *Culex quinquefasciatus*, 50 to 70 per cent infected, per bird) produces a far more virulent infection with exoerythrocytic forms and a fatality rate of about 90 per cent. The 3C blood induced infection is as severe as the sporozoite induced 3H2-1, and abundant exoerythrocytic forms are demonstrable when the infection has developed. The fatality rate of this strain with blood induced infection is 80 per cent. These strain differences are mentioned in particular, because it will become evident that they have a very definite relation to the results following treatment described below.

SN 8557 is described as 2-[3-(decahydro-2-naphthyl)propyl]-3-hydroxy-1,4-naphthoquinone, and probably consists of a mixture of four isomers with cis forms predominating (Fieser, 1946). For peroral administration the drug was prepared as a 0.75 per cent solution in olive oil and given twice per day in volumes adjusted to the individual weights of the birds, at the rate of 0.2 ml. per 20 g. This amounts to 150 mg./Kg.-day, and may be considered to be the maximum tolerated dose for the canary. Of 40 birds in the groups scheduled for 15 days treatment, 4 died during the period of treatment. None of those treated for 5 and 10 days died during the period of treatment.

With each of the eight types of infection (table 1) different groups of animals received 5, 10 or 15 days of treatment as outlined above. It was originally planned to have 5 birds in each group but 4 died during treatment, as mentioned

¹ The work described in this paper was done under a contract, recommended by the committee on Medical Research, between the Office of Scientific Research and Development and the University of Texas. The authors acknowledge the assistance of Cora Alice Taylor.

above, and 6 others died before sufficient information could be obtained as to whether or not they were cured and are therefore not included in the data presented. By way of explanation it may be mentioned that all blood induced infections were established by intravenous injection of a half million parasites. In group 4 the blood was taken from a sporozoite induced infection, but in the other groups the donors were from long lines of blood transfers. The infections in group 5 were induced by intravenous inoculation of sporozoites from *Culex quinquefasciatus* as mentioned above. Inspection of table 1 reveals the objective of the experiment, which is to determine the curative effect of SN 8557 in relation to the phase of infection (early, acute, latent), the kind of infection (blood or sporozoite induced), the strains, and the length of the period of treatment.

The results are recorded in table 1 as the ratio of the number of birds cured to the number of birds in each group. All the birds in group 1 which received treatment beginning the same day they were inoculated with strain 3H2-1 were

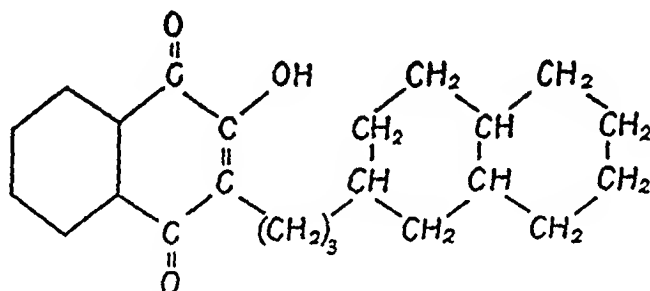


Fig. 1. SN 8557. 2-[3-(decahydro-2-naphthyl)propyl]-3-hydroxy-1,4-naphthoquinone.

cured, whether the drug was continued for 5, 10 or 15 days. It should be mentioned that one bird not included in the tabulated data received three doses of the drug after inoculation (1.5 days of treatment) and was not cured. The curative effect is therefore not a rapid killing of the injected parasites. When treatment is delayed for 3 days, allowing the infection to become well established as in group 2, the proportion of cures is poor until treatment has continued for 15 days. Similar experiments with the more virulent 3C strain (groups 6 and 7) yielded fewer cures as compared with the 3H2-1 strain, except when treatment was begun the day of inoculation and continued for 15 days. It is worth noting that there were fewer cures in group 7, with treatment begun during the acute infection of the virulent strain, than in any other group.

The results of administering the drug during latent infections of various types reveal a number of points of interest. From the tabulated data it appears that sporozoite induced 3H2-1 (group 5) and blood induced 3C (group 8) are cured with greater frequency than the milder blood induced 3H2-1 (group 3). The apparently anomalous result of only 2 cures in 12 birds as compared with the much higher proportion of cures in the other groups might be explained by a consideration of the age of the infections. It should be mentioned that the infections in group 4 represent one blood transfer removed from sporozoite

induced infection. The actual duration of latency for the several types of infections is not available, but the periods of time from inoculation to the experiment are as follows: group 3, 29 days; group 4, 35 days; group 5, 54 days; group 8, 95 days. If it be granted that the mild blood induced 3H2-1 in group 3 is cured with greater frequency than the infections in group 4 in spite of the shorter duration of infection, it seems not unlikely that the lesser frequency of cure in group 4 could be related to the shorter duration of infection as compared with groups 5 and 8. It is inconceivable that these infections are more resistant to

TABLE 1

Cures effected by SN 8557 (150 mg./Kg.-day) in various types of infections of Plasmodium cathemerium in the canary

GROUP	STRAIN	INFECTION INDUCED BY	PERIOD OF INFECTION AT TIME OF TREATMENT	DURATION OF TREATMENT IN DAYS			TOTALS
				5	10	15	
				<i>No. cures/No. birds</i>			
1	3H2-1	blood	day of inoculation	5/5	5/5	5/5	15/15
2	3H2-1	blood	third day after inoculation	2/5	0/5	4/5	6/15
3	3H2-1	blood	latent	2/5	2/4	4/5	8/14
4	3H2-1	blood from sporozoite induced-infection	latent	0/5	1/3	1/4	2/12
5	3H2-1	sporozoites	latent	3/4	4/5	4/4	11/13
6	3C	blood	day of inoculation	0/5	2/4	4/4	6/13
7	3C	blood	third day after inoculation	0/5	0/5	1/4	1/14
8	3C	blood	latent	3/5	4/4	5/5	12/14
Totals				15/39	18/35	28/36	61/110

cure than sporozoite induced infections. The explanation for this apparent inconsistency therefore rests on the theories that the regular blood induced 3H2-1 infections are more easily cured, and that the older latent sporozoite induced 3H2-1 and blood induced 3C infections are more easily cured because of the duration of latency, or on circumstances of which we are unaware.

To determine whether absolute cure had been effected, we drew 0.3 to 0.5 ml. of blood from each treated bird, and inoculated this intravenously into new birds on the 7th to 10th days after cessation of treatment (first transfer, table 2). The recipients were examined for a period of two weeks and if positive no further investigation was indicated as it was concluded that the "original" bird was not

cured. If negative, the recipient was tested about two weeks later for susceptibility by intravenous inoculation of known infected blood. Every reported negative transfer in table 2 was thus confirmed by susceptibility about one month after cessation of treatment. It is noteworthy that two weeks' negative blood examination of all the 157 recipients was not reversed by immunity to the challenge inoculation. Second transfers were similarly carried out for those original birds which had yielded negative transfers previously. For the birds which had received 15 days treatment third transfers were performed in the same manner. Following the three negative transfers these 28 birds were then challenged with intravenous inoculation of 10 million parasites (3H2-1) and developed acute infections. The remainder of the original birds were then similarly challenged and all but one were susceptible.

The one exception was in group 3 (table 1) and had received 5 days of treatment. Negative transfers on the 8th and 21st days after cessation of treatment

TABLE 2
Criteria of cure

FIRST TRANSFERS	SECOND TRANSFERS			THIRD TRANSFERS	CHALLENGE OF ORIGINALS
Days after cessation of treatment					
7 to 10	21	25	28	143	146 to 149
-:+	negative:positive			-:+	susceptible:immune
19:20	16:3				15:1
28:8		28:0		28:0	28:0
20:15			18:2		18:0
Totals	Totals			Totals	Totals
-:+	-:+			-:+	susceptible:immune
67:43	62:5			28:0	61:1

were confirmed by susceptibility of the recipients. The persistence of a marked immunity in this one bird 164 days after treatment when all of 60 others were susceptible to reinfection led us to the conclusion that the bird harbored a latent infection. The purpose of presenting the data in table 2 is to illustrate the results of tests for persistent infection in relation to the time after treatment. On the 7th to 10th days after treatment transfer of 0.3 to 0.5 ml. blood intravenously revealed infection in 43 birds but failed to reveal it in 6 others which had latent infections as judged by later information. On the 21st day after treatment 3 of these 6 were shown to be infected and 1 was missed. On the 28th day the remaining 2 were demonstrated to be harboring latent infection. No infections were missed with transfer of blood after the 25th day following the cessation of treatment. With a few similar experiences following administration of other drugs we have considered negative results of blood transfer unreliable when performed under 28 days after cessation of treatment.

Three other naphthoquinones were tested briefly on a schedule similar to group 1 for 4.5 days, and failed to effect cure. They are SN 12,320 which has the same formula as SN 8557 but is believed to consist chiefly of the trans forms;

SN 5090, the cyclohexylpropyl derivative; and SN 5949, the methyl octyl derivative. The therapeutic and prophylactic tests with these compounds are reported in the Survey.

DISCUSSION

The results of therapeutic and prophylactic tests with SN 8557 are published elsewhere (Survey of Antimalarial Drugs, 1941-1945), but they may be mentioned here briefly for comparison with the results of the curative tests. With strain 3H2-1, the therapeutic tests yielded a quinine equivalent of 1, that is, 0.24 mg. per day of both SN 8557 and quinine base produced approximately the same reduction of parasitemia. Prophylactic tests with sporozoite induced infections of the same strain were carried out on 4, 10, and 16-day schedules of treatment with 150 to 200 mg./Kg.-day, and failed to protect the birds completely although the first appearance of parasites in the blood was delayed a few days beyond the period of treatment. Both types of effect, therapeutic and prophylactic, may therefore be considered moderate as compared with other drugs. Other drugs have far higher quinine equivalents or greater prophylactic activity and no curative effect.

The question arises as to what may be considered a test for curative effect. If one assumes that the term "curative" may be applied properly only when treatment is begun after infection in the animal has become well established, then the procedure as used in group 1 may be considered as a curative test only qualifiedly. We use the term here in this sense because the results of the 4 to 5 day treatment begun the day of inoculation have corresponded with the results of 14 to 15 day treatment during latent or chronic infections with all the drugs we have tested. With a smaller inoculum, or a less virulent strain, or intramuscular and intraperitoneal modes of inoculation it may be anticipated that the short term procedure would eradicate or prevent infection far more frequently than with the long term procedure with well established infection. Under such circumstances the procedure could not be considered as a curative test. It is fortunate that the terminology in the pertinent literature is not confused, for Manwell (1930, 1932, 1933 and 1934) and Tate and Vincent (1933) use the term "sterilized" for eradication or prevention of infection by treatment during the incubation period (or acute infection) in avian malaria, whereas Coggeshall (1938) and Maier and Coggeshall (1944) use "cure" for eradication of infection by treatment during acute or chronic infection of *Plasmodium knowlesi* in monkeys.

The different response of the two strains in these experiments is in keeping with other tests and other drugs as well. The 3C strain requires greater amounts of quinine, quinacrine and pamaquine than does the 3H2-1 in the therapeutic test commonly used for determining the minimum effective dose or the quinine equivalent. This may well be related to the development of exoerythrocytic forms in 3C and the absence of them in 3H2-1. The latter strain we consider to be an exclusively erythrocytic infection when induced by blood inoculation.

The duration of immunity to *Plasmodium cathemerium* in canaries following cure was not known at the time of reinoculation in these experiments. Sus-

ceptibility about 5 months after treatment therefore is evidence for both loss of infection and loss of immunity. Whether there remains a degree of immunity comparable to that in monkeys 1 year after cure (Maier and Coggeshall, 1944) will be the subject of further investigation. It may be stated definitely, however, that there is no immunity comparable to that observed by Taliaferro and Taliaferro (1929) and Gingrich (1932) when latent infection is present.

SUMMARY

1. SN 8557, a 2-[3-(decahydro-2-naphthyl)propyl]-3-hydroxy-1,4-naphthoquinone, in doses of 150 mg./Kg.-day for 5, 10 or 15 days cured 61 of 110 canaries with various types of *Plasmodium cathemerium* infections. Administration of the drug for 15 days cured 28 of 36 birds (78 per cent) including some with latent sporozoite induced infections.

2. Acute infections of the exclusively erythrocytic strain 3H2-1 are more susceptible to cure than the 3C strain in which both erythrocytic and exoerythrocytic development occur. In latent infections, susceptibility to cure appears to be related to the duration of latency as well as to the strain.

3. A schedule of short term treatment (4 to 5 days) begun the day of inoculation (0.5×10^6 erythrocytic parasites intravenously) with strain 3H2-1 is a fair indicator for curative effect in latent infections, whether blood or sporozoite induced, or of a more virulent strain.

4. The duration of an efficient immunity to *P. cathemerium* in canaries is less than 5 months following cure.

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EXPERIMENTAL CHEMOTHERAPY OF AMEBIASIS¹

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The clinical control of amebic infection in man is complicated by two therapeutic factors as yet unsolved. For the treatment of the fulminating, dysenteric phase of the disease, emetine, which is a cardiac poison, has not been supplanted by any agent. Likewise, in amebic hepatitis and in abscess of the liver, emetine is still relied upon to control clinical symptoms, and yet alone it is not an effective amebicide. For the treatment of the chronic, resistant cases of amebiasis, carbarsone either alone, or in combination with one of the halogenated hydroxyquinolines, does not clear the bowel of amebas. No other chemical type is currently available for combatting *E. histolytica* infection in these resistant cases.

During the past two years we have restudied the chemotherapy of amebiasis with the hope of finding more effective chemical types of agents. Emphasis has been placed on the following aspects:

- (1) The pathogenesis of the disease in monkeys and man.
- (2) The utilization of macaques as experimental subjects.
- (3) The elaboration of more precise methods of diagnosis and follow-up of treated subjects.
- (4) The initiation of more uniform technics for *in vitro* testing of proposed new agents.
- (5) The development of synthetic media to provide the possibility of exploring the mechanism of biologic antagonism.
- (6) The attempt to explain possible mechanisms of action of anti-amebic agents.
- (7) To relate these studies to clinical control of amebiasis.

The pathogenesis of amebiasis, as visualized by Ash, Spitz (1) and others has been reviewed by Bostick (2). While the cause of amebic invasion is not known, it is influenced by: host resistance, pathogenicity of the organism, and the tissue environment of the colon. Local tissue cytolysis and motility of the trophozoites aid in penetration of the mucosa. This occurs between the gland openings, and occasionally down the mucosal gland. Chronically infected monkeys rarely exhibit amebas beneath the submucosa. In the cyst passer or so-called "carrier state" in man, minute, superficial lesions are found. It is difficult to correlate extent of lesions with diarrhea or other acute symptoms of amebiasis.

¹ With the technical assistance of Alvin S. Hambly Jr., Bertha Gardiner and Elsa Zitcer.

The work described in this report was done under a contract recommended by the Committee of Medical Research, between the Office of Scientific Research and Development, the Surgeon General's Office, United States Army Service Forces, and the University of California.

The characteristic lesions occurring in the cecum and rectum have been described (1, 2, 3). For pathologic diagnosis, it is significant that trophozoites lie at the margin of ulcers between viable and non-viable tissues. Unless secondary infection with bacteria has occurred there is little evidence of inflammatory reaction. Amebas may lie dormant for indefinite periods, without producing clinical manifestations. There may be no visible lesions unless colonization of amebas occurs.

In the systemic phase, the amebas may enter portal venules and lymphatics. They may reach the general circulation from colonies established in the liver. Spread throughout the body is accomplished by factors not clearly understood. With extra-colonic involvement, hepatic invasion is most frequent. Clark (4) has reported that fifty-five per cent of a series of autopsies had liver lesions. The lungs may become infected by direct extension from below the diaphragm. Brain abscess and other complications are rare occurrences. Perforation of the intestine has been observed in from ten to twenty per cent of autopsies. Less than three per cent have been reported clinically.

The pathology of natural amebic infection in macaques was reported a year ago before this society (3). The usefulness of monkeys as experimental subjects may be summarized as follows:

MONKEYS AS EXPERIMENTAL SUBJECTS

Macaques are infected naturally with *E. histolytica* similar to those found in man. Tissue invasion occurs: cysts and motile forms are found in stool specimens.

Monkey infection resembles chronic human amebiasis more closely than experimental amebic infections in other animals.

Like human disease in temperate zones, monkey infection is resistant to therapy. Agents effective in monkeys are useful in man. Safety-margin of active amebicides should be determined in chronically infected macaques: direct correlation has been found with subsequent studies in man.

The elaboration of more precise methods of diagnosis and follow-up of treated subjects has been stressed (5). Diagnosis, while dependent upon the awareness of the physician, is essentially a laboratory problem. It should be entrusted only to trained personnel who are familiar with the parasitology of the intestinal tract and who appreciate the importance of the necessary prerequisites demanded for the establishment of a correct diagnosis of amebiasis. Microscopic examination of the cytology of the freshly passed stool may at times provide presumptive evidence in the more acute phases of the disease.

In the present study, iron-hematoxylin stained smears have been relied upon for pre-treatment examinations as well as for those performed during the arbitrary three months follow-up period. Lincicome (6) and others have shown that amebas appear in cyclic showers. It has been our practice to examine one specimen daily for six consecutive days. The first series of examinations is begun the day after completion of therapy; six weeks later a second series is examined;

with a third and final series in another six weeks. Thus, if careful search of eighteen specimens over a three months period fails to reveal pathogenic amebas, the subject is declared free of infection. To reduce the possibility of reinfection, in the laboratory, monkeys are isolated in cages with "false bottoms" which are scoured frequently.

The initial screening of proposed agents *in vitro* has been refined by the use of a single strain of *E. histolytica* associated with a single strain of organism "t" (7). Because some agents, especially emetine and the crystalline products of chaparro amargoso, require long exposure to exhibit activity, tests were continued for forty-eight hours at 37°C.

We have the definite conviction that truly amebacidal rather than amebastatic activity should be regarded as the criterion of effectiveness. As a check on the end-point, in the *in vitro* test, subculture is made routinely, in order to determine whether viable forms are present. This technic has been further improved by the use of a liquid liver medium which avoids possible absorption of potentially effective agents by the protein contained in the conventional egg-slope medium. Also, an essentially synthetic medium has been developed which is water clear. Except for rice starch granules and nucleic acid, it contains only synthetic elements (8). This medium affords the opportunity for the development of biologic antagonists which may prove to be of therapeutic importance. Of 234 agents examined, those found to be more active than emetine in one of the media used, are listed in table 1. Thus, fifteen new amebacides proved to be sufficiently promising to warrant trial *in vivo*. In addition, the most active isothiurea and least toxic acridine were included to explore different chemical classes. Related chemical types, more toxic for mammals, are not included.

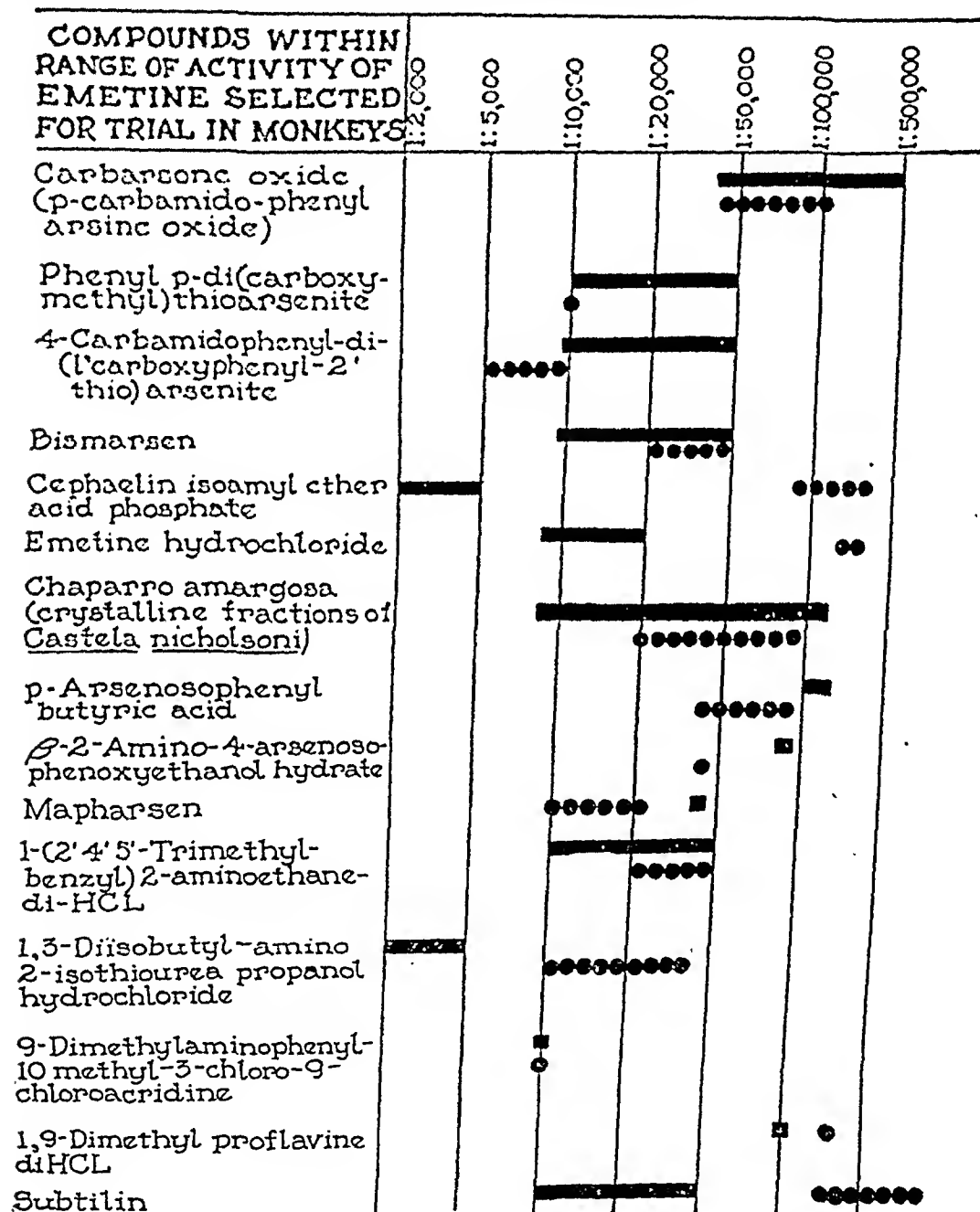
In an attempt to weigh the toxicity for host tissue cells, *in vitro* exposures of rabbits' white cells were made. After thirty minutes, the effects of representative chemical types, on preliminary trial, were observed. With agents such as emetine and chaparro amargoso, believed to be amebacidal only after long exposure, no untoward action on white cells was noted. On the other hand, representative acridines inhibited the motility of white cells at amebacidal concentrations *in vitro*. These dyes, on the basis of mammalian toxicity tests, had a small margin of safety. Other active amebacides were not leukocidal in 1:500 to 1:1,000 concentrations. These included the three trivalent arsenicals tested, as well as subtilin. Several chemical types are being studied with the hope of using this *in vitro* test as an aid in anticipating the level of toxicity for the host's tissues.

All but two of these agents were given orally or parenterally in maximum tolerated doses to thirty-one infected macaques. The period of daily treatments varied from five to thirty days. In every animal an electrocardiogram and blood urea and bromosulfalein tests were made before and after therapy. Eight drugs, partially effective in clearing monkeys caused toxic effects believed to be due to the agent under trial. One agent, a proflavine derivative not tried in these animals, was employed without success in man. One other agent, subtilin, the only antibiotic we have found to be effective against amebas, was not avail-

TABLE 1

Amebacidal activities in vitro of some representative agents

In egg slope media: ■■■■■. In liquid liver media: Length of line indicates range within which minimum amebacidal concentration occurs.



able in sufficient quantity until recently to permit *in vivo* trial. The results of trials of new agents, in comparison with emetine, are summarized in table 2.

TABLE 2

Experimental therapy of amebiasis
 Period of clearance in naturally infected macaques

⁺ COMPOUNDS GIVEN ORALLY	30 days	60 days	90 days	120 days
Carbarsone oxide (p-carbamidophenyl arsine oxide) 11-40mgm/Kg. x 5-30 days	=====	=====	=====	=====
Phenylurea p-di(carboxymethyl)thioarsenite 25mgm/Kg. x 7 days	=====	=====	=====	=====
4-Carbamidophenyl-di-(1'-carboxyphenyl-2'-thio) arsenite 25mgm/Kg. x 8 days	=====	=====	=====	=====
Bismarsen, 30mgm/Kg. x 7 days	=====	=====	=====	=====
Cephaelin iso-amylether acid phosphate, 2mgm/Kg. x 10 days	=====	=====	=====	*
Emetine hydrochloride 10(oral); 3(H)mgm/Kg. x 5 days	===== (H)*	=====	=====	=====
Chaparro amargosa (Crystalline fractions of <i>Castela nicholsoni</i>) 3-50mgm./Kg x 5 days	===== *	=====	=====	=====
p-Arsenosophenyl butyric acid 3mgm/Kg. x 10 days	===== (IV)*	=====	=====	=====
p-2-Amino-4-arsenosophenoxyethanol hydrate 5mgm/Kg X 5 days	===== (IV)*	=====	=====	=====
Mapharsen, 10mgm/Kg. x 5 days	===== (IV)*	=====	=====	=====
1-(2'4'5'-Trimethylbenzyl)-2-aminoethane-di-HCL 30mgm./Kg. x 7 days	=====	=====	=====	=====
1,3-Diisobutylamino-2-isothiouraea propanol hydrochloride, 10mgm/Kg. x 5 days	===== *	=====	=====	=====
9-Dimethylaminophenyl-10-methyl-3-chloro-9-chloro-acridine. HCL, 50mgm/Kg. x 7 days	===== *	=====	=====	=====

¹ Agents as active *in vitro* as emetine, and having relatively low toxicity for mammals are included. Each line represents one animal. + Unless otherwise indicated; * Evidence of Drug Toxicity; (H) Subcutaneously; (IV) Intravenously.

Of the remaining four arsenicals, carbarsone oxide (p-carbamido phenyl arsine oxide) proved most useful. It has been given subsequently to five patients with

resistant or chronic amebiasis, and in four of these, stools have been cleared during the follow-up period. A typical case history is as follows:

CASE HISTORY

L. P.—Male, age 72, married, wt. 72 Kg. Acquired infection on Corregidor in 1902. Developed dysentery and lost 10 Kg. in weight. Hospitalized—ten months where diagnosis established. Given Ipecac until symptom-free. Apparently well until 1935 when bloody stools developed. In 1944, lost weight—12.5 Kg. in 18 months; had an intestinal upset. In 1945, chills, fever, bilirubinuria, intermittent icterus and mild right epigastric pain: *Chronic cholecystitis with cholelithiasis* was diagnosed. At operation liver was “enlarged with whitish patches on surface.” Nov. 1945—*E. histolytica* found in stools. Proctoscoped: No lesions were observed. Tests of liver and kidney function were within normal limits. R̄ carbarsone oxide, 30 mgm. was given orally three times daily after meals for ten days. Stools cleared (*S series in 6 months*); has been clinically well for six months.

No ill effects have followed controlled use of this arsine oxide in man. It should be noted, however, that this is the first time that an agent of this type has been given orally. In tolerated amounts, no pathologic effects were seen in animals. From our studies in rats, however, one might expect topical effects on the gastric mucosa from toxic amounts if adequate safeguards are not employed. These include the use of the drug in enteric coated tablets in single doses not exceeding 30 mgm. which are given immediately after food has been taken. Otherwise, patients may complain of epigastric distress. Since the margin of safety is not likely to be as great as with its pentavalent arsenical analogue, carbarsone, control of total dosage and time of application are most important. The pharmacology of this agent will be reported in detail elsewhere. A preliminary report on this aspect of the study has been made (9).

Two other trivalent arsenicals, which contain sulfur, were completely effective in macaques. Phenyl-urea p-di-(carboxymethyl)-thioarsenite has a lower level of systemic toxicity than carbarsone oxide. The thioarsenite is being investigated further since it may ultimately be shown to cause less damage to the gastric mucosa at acutely toxic levels. Monkeys remained free of ameba during the follow-up period after seven daily oral doses of 25 mgm. per kilo. No ill effects have been observed. The other agent, 4-carbamidophenyl-di-(1'-carboxyphenyl-2'-thio)-arsenite, was equally effective in monkeys in the same dosage given over an eight day period.

When additional pharmacologic data are available, it is possible that one of these sulfur containing trivalent arsenicals may prove to be therapeutically useful. Cohen, et al. (10) have shown that *in vitro* the thioarsenites slowly liberate arsenoxide which acts against another protozoan, the trypanosome. It is believed that the greater therapeutic usefulness of thioarsenites is due to slow formation of arsenoxide that is thought to occur in the blood stream. Partial hydrolysis has been demonstrated within thirty minutes; this continues over an extended period. Thus, we have a less toxic agent for the host's tissues. The active form is gradually dispersed until full therapeutic effect is accomplished.

Another chemical type, tested in monkeys was 1-(2', 4', 5'-trimethylbenzyl) 2-aminoethane dihydrochloride. Initially, when screened *in vitro* it appeared to be more active than emetine. On the basis of this test, two monkeys were given 30 mgm. per kilo daily by mouth for seven days. The drug was well tolerated but apparently only temporary clearance of the stools was accomplished. On re-examination of the drug *in vitro* it was found to be less effective than emetine. Thus, the final check of *in vitro* activity correlated with the *in vivo* trial. One other drug type, an isothioureia, was less effective than emetine *in vitro* and in monkeys it cleared the stools only during the period of treatment. The latter proved toxic in one animal.

It should be noted that the pentavalent arsenical carbarsone was found to be active only when given in large amounts to monkeys (11). Despite this, carbarsone is the most active of the currently available amebicides. In anticipation of continued trial of new amebicides in man, these facts should be kept in mind. While we feel that considerable correlation exists between results in monkeys and the present modified *in vitro* testing technic, it remains to be shown whether this correlation extends finally to successful application of new agents to man.

An attempt has been made to explore possible mechanisms of action of anti-amebic agents. It should be recognized that the effective agent, to be completely useful, must kill amebas, not only in the lumen of the bowel, but in the tissues of the host as well. Dobell and Laidlaw suggested in 1926 that emetine interfered with the multiplication of amebas. James working in Panama in 1913, reported on "The Effects of Certain Drugs on the Pathogenic *Entamoebae* of the Human Intestines" in the first volume of the American Journal of Tropical Diseases and Preventive Medicine (pg. 431). Thus, within a year of the initial use of emetine in man, and within six months of James' first experience with the drug, an effort was being made to explain its mode of action. Illustrations which accompanied his report revealed that the administration of emetine apparently caused a marked granular degeneration of the nucleus of the ameba, together with reticulation of the cytoplasm. The nuclear membrane was less distinct and after larger amounts of emetine had been given the ameba contained inclusion bodies. So far as we know, little has been added to our knowledge of the action of emetine on the ameba since these early observations. The only other evidence of direct amebicidal activity is the present demonstration of the surface lowering action of the antibiotic subtilin (12). Rupture of the cell wall occurred when the amebas were exposed to effective concentrations of subtilin.

Some possible mechanisms of action which may be considered, of various active chemical and biologic types, may be summarized as follows:

POSSIBLE MECHANISMS OF ANTI-AMEBIC ACTION

Emetine. Interferes with multiplication of trophozoites (Dobell and Laidlaw) (13); causes degeneration of nucleus and reticulation of the cytoplasm which contains inclusion bodies (James).

Halogenated hydroxyquinolines. Attack enzymes or halogenate proteins of cells.

Arsenicals. Active forms are phenyl arsine oxides with affinity for red cells and other host cells; *when greater affinity for amebas exists* drugs become clinically useful. They combine with thiol ($-SH$) groups in enzyme systems of cells.

Acridines. 1,9 Dimethyl proflavine was more active *in vitro* than *in vivo*. Probably these dyes combine with acidic groups in enzyme systems of cells.

Subtilin. Lowers surface tension—*causes rupture of membrane of ameba*—first evidence of direct amebacidal activity *in vitro*.

Thio-ureas. Probably act by inhibiting peroxidase.

Required nutrients. Determined by growth in synthetic media—possibility of chemical modification offers a rational approach to chemotherapy.

The authors wish to acknowledge the help and advice of Florence H. Wood, Gordon A. Alles, Victor P. Bond, and Y. C. Chin.

SUMMARY

- (1) The pathogenesis of amebiasis is discussed.
- (2) Comparative amebacidal tests *in vitro* and *in vivo* have been made, utilizing improved appraisal technics.
- (3) The development of an essentially synthetic medium may lead to a more rational approach to the chemotherapy of this disease.
- (4) The mechanisms of anti-amebic action have been demonstrated for two of the various chemical types available.
- (5) Three trivalent arsenicals (two containing sulfur) have proved of value in trials in macaques, and one has been effective in initial trials in man.

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THE REACTION OF WOOLLY OPOSSUMS (*CALUROMYS LANIGER*) TO YELLOW FEVER VIRUS*

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INTRODUCTION

Interest in animals of the order Marsupialia in connection with the epidemiology of jungle yellow fever was stimulated by the work of Bugher and his colleagues (1, 2). Although these investigators found that marsupials of a number of different species were susceptible to infection with yellow fever virus, most of their work was confined to *Didelphis marsupialis*. Bates (3) has reported the results of his studies of yellow fever in two other species of marsupials. He found that a considerably higher per cent of *Metachirus nudicaudatus* than of *Metachirops opossum* circulated virus following inoculation. Judging from the published reports, *Metachirus nudicaudatus* would be considered to be more susceptible to the virus than *Didelphis marsupialis*. A report by Laemmert (4) on the susceptibility of a number of species of marsupials, generally confirms the work of the authors mentioned above and includes information on several previously unstudied species.

Since Bugher *et al.* (2) have stated that marsupials may play an active rôle in the maintenance of jungle yellow fever, it is important to learn as much as possible concerning the reaction of these animals to yellow fever virus.

The study here reported was undertaken to obtain more precise information about the immune reaction of the woolly opossum following inoculation of yellow fever virus. We include in the report some information that has been obtained relative to the susceptibility of this species.

MATERIALS AND METHODS

Animals. A specimen typical of the woolly opossum employed in these experiments was sent to Dr. G. H. H. Tate of the American Museum of Natural History and was identified by him as *Caluromys laniger*. A discussion of the taxonomy of this species, together with an excellent picture, may be found in the article by Bates (3). The animals we used were caught in Volcanes, Villeta, and Restrepo. The first two localities are in the Magdalena valley in the State of Cundinamarca, and Restrepo lies at the foot of the eastern range of the Andes, some 20 kilometers northeast of Villavicencio, in the province of Meta. These three localities are considered to be in endemic yellow fever zones, and cases of the disease have been discovered near Restrepo and Volcanes within the past few years.

The animals were kept in individual cages and were maintained on a diet of vegetables, fruits, and milk. The mortality in animals newly arrived in the

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the Institute of Special Studies "Carlos Finlay", maintained by the Ministry of Labor, Hygiene and Social Welfare of the Republic of Colombia and the International Health Division of The Rockefeller Foundation.

laboratory was quite high, but those that survived the first few weeks ordinarily lived quite a long time. All of the animals were marked by clipping their toes according to a key that was recorded on each animal card. This method has been found to be more satisfactory than the metal tags usually employed, especially when the animals are to be kept for long periods of time.

Virus strains. Several strains of yellow fever virus of human and mosquito origin were employed in the susceptibility experiments. Data on each strain are presented below.

Asibi: a strain of human origin isolated by Stokes, Bauer, and Hudson (5) that has been maintained in continuous passage in rhesus monkeys.

Isabi: a strain isolated by the intracerebral inoculation into white mice of a suspension of wild-caught haemagogus mosquitoes. It was passed once more intracerebrally in white mice and then inoculated into a rhesus monkey, the dried serum of the latter serving as the source of infectious material.

Tambo-redondo: a strain isolated by the intracerebral inoculation into white mice of a suspension composed of a mixture of wild-caught *Aedes dominicii* and *Aedes leucocelaenus* mosquitoes. The infectious material employed in these experiments was a suspension of dried brain of the fifth mouse passage.

Volcanes: a strain isolated in a rhesus monkey infected by the bite of wild-caught haemagogus mosquitoes. The strain was then passed once intracerebrally in white mice, the dried brains of this mouse group serving as the source of infectious material.

Gordillo: a strain of human origin isolated in white mice. Dried brains of the sixteenth mouse passage group served as infectious material.

Chichimene: a strain isolated in a rhesus monkey infected by the bite of wild-caught haemagogus mosquitoes. Following a single passage in white mice, the strain was reinoculated into a rhesus monkey, the dried serum of the latter serving as the source of virus.

Further details attending the isolation of these strains may be found in the literature (2, 6).

Susceptibility tests. Our method of performing the susceptibility tests was essentially the same as that employed by other investigators. The experiments described here were carried out while we were working in different laboratories, and since the routines adopted for testing for circulating virus differed slightly, each will be briefly described.

Following a preinoculation bleeding, all animals included in the experiments were injected subcutaneously with either 0.5 cc. or 1.0 cc. of the infectious suspension. At the time of inoculation, the infectious suspension was titrated in ten-fold dilutions, intracerebrally, in adult white mice, in order to determine the amount of virus received by each opossum. By one method, the opossums were tested for the presence of circulating virus by inoculating adult white mice intracerebrally with a 1:3 dilution in saline of the whole blood, drawn from either the heart or the femoral vein. Animals tested in this manner were bled from the second through the tenth day following inoculation. By the other technique, the animals were bled from the heart from the second or third day

following inoculation through the fifth or sixth day and the serum was inoculated intracerebrally into adult white mice.

Occasionally a group of three-day-old mice was inoculated intracerebrally in parallel with the group of adult mice in order to compare the sensitivity of the two age groups to the virus. At least one specificity test was performed on the virus isolated from each opossum that circulated virus. A passage was also made, when possible, from each group of mice that showed signs of infection, in order to be sure that the illness was due to yellow fever virus, which would be evidenced by the occurrence of the typical disease in the passage group of mice.

The opossums were then bled at intervals to obtain serum to be examined for the presence of specific neutralizing antibody.

Detection of specific antibody. All tests for the presence of yellow fever antibody were performed by the intracerebral neutralization technique (7) as modified by Bugher (8). Whenever possible, the pre- and postinoculation sera were examined in the same test. The interpretation of the results will be discussed in another section of the paper.

Calculation of titers. All titers were estimated by the fifty per cent end-point method described by Reed and Muench (9).

EXPERIMENTAL

Since more than half of the animals received Chichimene virus, the results of the susceptibility tests with this strain will be presented separately. The Chichimene strain was favored for two reasons: first because it was known to be highly neurotropic for white mice, thus making its detection in the circulation more certain; secondly, because preliminary experiments revealed that a high proportion of animals circulated virus following the inoculation of relatively small doses, and since the principal object was to study the immune reaction, this was a desirable characteristic.

Because of some mortality among the animals during the course of the experiments, it has been necessary to select animals to be considered in this report rather than to include the results obtained on every animal inoculated. Logically, the animals in which circulating virus was detected have been included, irrespective of the time of death. All animals that died during the period of bleedings for the detection of circulating virus, and had not circulated virus at any time, were excluded. The basis for exclusion, however, depended on the particular routine employed for the detection of circulating virus. For example, an animal dying on the seventh day after inoculation and without circulating virus was excluded from consideration if that animal was tested for susceptibility according to the first technique described in the section *Materials and Methods* but was included if tested by the second method. However, only a small number of animals have been excluded on this basis.

Only animals with negative preinoculation protection tests have been considered in this report.

Susceptibility of Caluromys laniger to the Chichimene strain of yellow fever virus. Forty-three woolly opossums, in groups of two to six animals, were inoculated,

subcutaneously, with widely varying doses of Chichimene virus. Of the entire group, 31 (72 per cent) were found to be susceptible to this strain, as proved by the isolation of yellow fever virus from the blood stream several days after inoculation. The results of these experiments are summarized in table 1. The distribution of the susceptible animals would seem to indicate that the susceptibility was independent of the size of the infectious dose. In order to test this conclusion, a titration of the same strain was made in a group of 20 opossums.

TABLE 1
Susceptibility of C. laniger to the Chichimene strain of yellow fever virus

NO. OF ANIMALS INOCULATED	DOSE OF VIRUS	NO. OF ANIMALS CIRCULATING VIRUS ON GIVEN DAYS AFTER INOCULATION*										TOTAL NO. OF ANIMALS CIRCULATING VIRUS	POSTINOCULATION NEUTRALIZATION TEST RESULTS				
		2	3	4	5	6	7	8	9	10	No. of animals bled		Virus in circulation		No virus in circulation		
													Pos.†	Neg.†	Pos.†	Neg.†	
6	76,600	—	4	3	4	3	1	—	—	—	4	6	4			1	1
2	24,000	0	1	—	—	0	0	0	0	0	1	2	1			1	
2	16,666	0	—	—	—	—	0	0	0	0	0	2†					
4	13,400	1	4	3	4	3	1	1	—	—	4§	2	2				
4	8,500	0	3	4	2	—	0	0	0	0	4	4	4				
4	8,333	3	3	2	2	0	0	0	0	0	3	1		1			
2	6,667	0	—	1	—	—	0	0	0	0	1	2	1			1	
4	4,400	0	1	—	—	—	0	0	0	0	1	4	1			2	1
5	2,550	0	3	2	4	—	0	0	0	0	5¶	4	3	1			
3	1,590	0	—	1	3	3	0	0	0	0	3¶	2	2				
3	1,000	0	1	1	—	0	0	0	0	0	1	3	1				2
2	708	0	2	2	2	2	0	0	0	0	2¶	1	1				
2	47	—	—	—	1	2	2	—	—	—	2	2	2				
43	Totals										31	35	22	2		5	4

* — signifies that the animals were bled but did not have detectable virus in the blood. 0 signifies that the animals were not bled. The figures indicate the number of animals that circulated virus on the given day after inoculation.

† The figures represent the number of animals whose sera gave the indicated response.

‡ The postinoculation protection tests gave unsatisfactory results.

§ Two of the animals did not survive for postinoculation bleedings.

|| Two of the virus-circulating animals and the one not circulating virus did not survive for postinoculation bleedings.

¶ One animal did not survive for the postinoculation bleeding.

Tenfold dilutions of an infectious suspension were inoculated subcutaneously into groups of five animals, the largest dose constituted 18,600 LD₅₀ for white mice and the smallest only 18.6 LD₅₀. The significant features of this experiment may be found in table 2. The results would appear to confirm the impression gained from the previous experiments, in that 15 of the 20 animals circulated virus, and the distribution of the susceptible animals was uniform throughout the range of virus dosage that was studied. However, the results of the reinoculation experiments made it necessary to modify this view.

Since a number of these animals were not bled after the sixth day, it is necessary to establish the significance of this method. Of the group of 63 opossums inocu-

lated with the Chichimene strain, 32 were bled from the second through the tenth day after inoculation. None of the animals that circulated virus in this group showed the *first* evidence of circulating virus after the sixth day.

Susceptibility of C. laniger to other strains of yellow fever virus. Table 3 presents a summary of the susceptibility experiments performed with strains of yellow fever virus other than the Chichimene strain. In all instances, less than 1,000 LD₅₀ for white mice were inoculated. Virus was recovered from the blood of 15 (52 per cent) of the 29 animals studied. In most experiments, the number of animals inoculated with a given strain was too small to be significant in studying the relationship of susceptibility to the strain of virus. However, the results of experiments with the Tambo-redondo strain and the Volcanes strain strongly

TABLE 2
Titration of Chichimene virus in C. laniger

NO. OF ANIMALS INOCULATED	DOSE OF VIRUS	NO. OF ANIMALS CIRCULATING VIRUS ON GIVEN DAYS AFTER INOCULATION*									TOTAL NO. OF ANIMALS CIRCULATING VIRUS	POSTINOCULATION NEUTRALIZATION TEST RESULTS				
		2	3	4	5	6	7	8	9	10		No. of animals bled	Virus in circulation		No virus in circulation	
													Pos.†	Neg.†	Pos.†	Neg.†
5	18,600	2	2	4	3	2	1	—	—	—	4†	4	3			1
5	1,860	—	3	2	4	2	1	1	1	—	4†	4	3		1	
5	186	—	2	3	3	3	2	2	1	1	4	5	3	1	1	
5	18.6	—	2	2	1	3	2	2	1	1	3	5	3			2
20	Totals										15	18	12	1	2	3

* — signifies that the animals were bled but did not have detectable virus in the blood. The figures indicate the number of animals that circulated virus on the given day after inoculation.

† The figures represent the number of animals whose sera gave the indicated response.

‡ One animal circulating virus did not survive for postinoculation bleeding.

suggested that such a relationship may exist. All of the eight opossums receiving the Tambo-redondo strain circulated virus, whereas virus was recovered from only two of nine opossums following inoculation of the Volcanes strain.

As stated in the section, *Materials and Methods*, the animals employed in these experiments were caught in three different areas. It was thought that since all of these localities were in yellow fever zones, the recent presence of yellow fever in one and not in the other might affect the proportion of susceptible animals, and thereby account for results such as were obtained with the Tambo-redondo and Volcanes strains. Specifically, virus had been isolated in the Volcanes area less than a year before the opossums from that locality were caught, but yellow fever has not been known to occur in the Villeta region during the past five years. Of the 92 animals that have been considered, 35 came from Volcanes and 37 from Villeta. Twenty-five of the Volcanes opossums and 24 of those from Villeta circulated virus.

As mentioned above, groups of three-day-old mice were occasionally inoculated in parallel with the adult mice. In some instances it was possible to isolate

virus in the baby mice when none of the corresponding adult mice showed signs of infection. In a few cases, the infection of baby mice was the only evidence of circulating virus obtained.

None of the animals dying during the course of the experiments presented any lesions, grossly or microscopically, suggesting that the death might be due to the yellow fever virus¹. A number of animals were found to be heavily infected with microfilaria, and this parasite may have been responsible for the death of these animals. Infection at the site of bleeding in many of the animals bled from the

TABLE 3
Susceptibility of C. laniger to different strains of yellow fever virus

NO. OF ANIMALS	STRAIN OF VIRUS	DOSE OF VIRUS	NO. OF ANIMALS CIRCULATING VIRUS ON GIVEN DAYS AFTER INOCULATION*										TOTAL NO. OF ANIMALS CIRCULATING VIRUS	POSTINOCULATION NEUTRALIZATION TEST RESULTS				
														No. of animals bled	Virus in circulation		No virus in circulation	
			2	3	4	5	6	7	8	9	10	Pos.†			Neg.†	Pos.†	Neg.†	
4	Volcanes	760	—	—	—	—	2	1	1	1	—	2‡	3	1				2
5	Volcanes	485	0	—	—	—	0	0	0	0	0	0	5			1		4
3	Asibi	711	—	—	—	—	1	1	—	1	1	2‡	2	1				1
2	Asibi	533	—	—	—	1	1	0	0	0	0	1	0					
4	Gordillo	330	—	—	1	1	1	—	—	—	—	1	4	1				3
3	Isabi	261	—	—	1	1	1	—	—	—	—	1	3	1		1		1
3	Tambo-redondo	249	0	—	1	1	2	—	1	—	—	3	3	3				
5	Tambo-redondo	100	0	4	5	5	0	0	0	0	0	5	5§	4				
29	Totals											15	25	11		2		11

* — signifies that the animals were bled but did not have detectable virus in the blood. 0 signifies that the animals were not bled. The figures indicate the number of animals that circulated virus on the given day after inoculation.

† The figures represent the number of animals whose sera gave the indicated response.

‡ One animal with circulating virus did not survive for the postinoculation bleeding.

§ The postinoculation neutralization test on one of the animals gave an unsatisfactory result.

femoral vein, and pericardial hemorrhage in those bled from the heart, were the common causes of death.

Titer of circulating virus. On several occasions, serum from animals infected with the Chichimene or the Tambo-redondo strains was titered in mice. The data are presented in table 4. It can be seen that no significant difference exists between the two strains in relation to the titer of virus in the infected animals. The highest titer obtained was 1/320, but the majority of titers were less than 1/10.

The immune reaction of C. laniger to yellow fever virus. The immune reaction of woolly opossums following inoculation of yellow fever virus has been studied

¹ The authors are indebted to Dr. Augusto Gast-Galvis for examination of the pathological material obtained from animals dying during the course of these experiments.

by two methods: chiefly by investigating the production and duration of specific neutralizing antibodies, and secondarily, by observing the response of previously inoculated individuals to large challenge doses of virus.

The first few tests on sera from animals that had circulated virus clearly demonstrated that the amount of antibody produced was frequently small. Thereafter, only small amounts of virus (30-80 LD₅₀) were employed in the neutralization tests.

As a consequence of the slight amount of antibody produced by some of the infected animals it was imperative to make careful studies of the behavior of normal opossum serum in the neutralization test. Unfortunately, it was not possible to examine the sera of all of the animals that circulated virus in tests employing small doses of virus because of the inherent variability of dried virus preparations. However, of the 61 animals that circulated virus, 41 had their

TABLE 4

Titer of yellow fever virus in the serum of infected C. laniger

ANIMAL NO.	STRAIN INOCULATED	DOSE OF VIRUS	VIRUS TITERS DAYS AFTER INOCULATION			
			3	4	5	6
3359	Chichimene	1,590		<1/1*	1/32	1/32
3360	Chichimene	1,590			1/42	1/6
3361	Chichimene	1,590			1/25	1/7
3371	Tambo-redondo	100	<1/4	<1/4	1/320	
3389	Tambo-redondo	100	<1/4	<1/4	<1/4	
3402	Tambo-redondo	100	<1/4	<1/4	1/32	
3403	Tambo-redondo	100	<1/4	<1/4	1/25	
3405	Tambo-redondo	100	<1/4	<1/4	<1/4	

* < Indicates that the titer was less than the dilution shown but nevertheless virus was present.

preinoculation sera examined in tests in which the virus dose was between 30 and 80 LD₅₀, the majority of the tests employing less than 50 LD₅₀. All of these sera gave negative results and the average survival time of the mouse groups in which they were tested was usually less than 5.5 days, the maximum observed being 6.5 days. The remaining sera were examined in tests employing only slightly larger doses and likewise gave consistently negative results. Several sera were examined in tests with between 12 and 16 LD₅₀ of virus. Although these tests were considered to be unsatisfactory because several of the human control sera gave survival ratios greater than 1/6, only one of the 10 preinoculation opossum sera showed evidence of protection, giving a 2/6 survival ratio. Actually, no evidence has been obtained to indicate that non-specific viricidal activity of the serum is a factor to be taken into account in the interpretation of the neutralization test results on sera of *C. laniger*.

The data on the neutralizing antibody response have been divided, for convenience, into two portions. First, a consideration of the more immediate

response, i.e., a study of the neutralizing antibody in sera obtained shortly after exposure to the virus; and secondly, a study of the duration of the neutralizing antibody in animals that were known to have circulated virus and to have developed these antibodies.

Since animals circulating virus often produced only small amounts of neutralizing antibody, a certain amount of difficulty was experienced in the interpretation of some of the results obtained on postinfection sera. Of the group of 61 woolly opossums that circulated virus, only 48 survived for postinfection bleedings. In table 5, the maximum survival ratios obtained in the neutralization tests performed on the preinoculation and postinoculation blood samples of these 48 animals have been presented. It is interesting to observe the similarity in distribution of these survival ratios to those obtained in tests with human sera following vaccination against yellow fever with the 17D vaccine strain (10). Soper and Smith (11) have found that some human postvaccinal sera give 1/6 and even 0/6 survival ratios with prolonged average survival times. Since this is infrequent with non-immune human sera, these authors have considered that

TABLE 5

The distribution of neutralization test survival ratios of pre- and postinoculation sera from infected C. laniger

TYPE OF SERUM	NEUTRALIZATION TEST SURVIVAL RATIOS*											TOTAL
	0/5	0/6	1/6	2/6	3/4	3/5	3/6	4/5	4/6	5/6	6/6	
Preinoculation.....	4	38	6									48
Postinoculation.....			3	3	1	2	3	4	9	10	13	48

* The figures indicate the number of animals whose sera gave the specified response.

the majority of such reactions indicate the presence of small amounts of antibody. The preinoculation sera of this group of opossums were consistently negative and the postinfection sera often reacted as weakly positive sera, so that it seemed reasonable to use somewhat the same criteria for interpretation that have been used for human postvaccinal sera. It has been considered here that a survival ratio of 2/6 should be the minimum necessary to be interpreted as evidence of neutralizing antibody. Although the three sera classified as "negative" in table 5, with survival ratios of 1/6 following virus circulation, all showed prolonged average survival times, especially when compared with the preinoculation serum samples, the finding of occasional normal sera with average survival times of greater than six days would make the interpretation of these sera as "positive" somewhat hazardous. This would be especially true because the experiments have been performed in order to establish criteria for the interpretation of serum samples obtained in the field, where often only sufficient serum for one test is available.

The most convincing evidence supporting the above-mentioned criteria for interpretation of 2/6 to 4/6 survival ratios as showing evidence of immunity is based on the reproducibility of the results and the fact that no animal with a

survival ratio of 2/6 or greater has circulated virus. Table 6 presents the results of several duplicate tests on sera with lower survival ratios. The small variations observed between the results of different tests were usually correlated with variations in the amount of virus used in each test; the lower survival ratios increased as the number of doses in the test decreased. An able discussion of many of the variables that must be considered in the interpretation of the intracerebral neutralization test in yellow fever may be found in an article by Bugher (8).

The protection test data on the animals discussed in the susceptibility studies, interpreted on the basis described above, have been included in tables 1, 2, and 3. It can be seen that the majority (94 per cent) of animals circulating virus later showed evidence of specific neutralizing antibodies. It can also be seen that

TABLE 6

Results of duplicate neutralization tests on postinoculation sera with low survival ratios

ANIMAL NO.	NEUTRALIZATION TEST RESULTS								
	First test			Second test			Third test		
	Survival ratio	MLD*	AST†	Survival ratio	MLD	AST	Survival ratio	MLD	AST
3317	4/6	31	9.0	3/6	85	8.7			
3317	4/6	31	8.7	2/6	85	7.8			
2691	2/6	160	7.7	6/6	195	10.0			
2709	1/6	144	8.0	3/6	85	8.2			
2709	1/6	144	6.2	2/6	85	8.0			
2714	1/5	144	7.6	1/6	139	7.0	2/6	68	7.5
2716	5/6	144	9.7	1/6	68	7.2			
2716	3/6	144	8.3	5/6	68	9.2			

* MLD signifies the number of fifty per cent mouse mortality doses employed in the neutralization test.

† AST signifies the average survival time of the mouse group.

several animals developed neutralizing antibodies without showing evidence of circulating virus. The interpretation of this finding will be discussed more fully in a later section.

Duration of specific neutralizing antibody. Animals were bled at various intervals following exposure to yellow fever virus, either to obtain preinoculation samples for the challenge experiments, or merely to acquire data on the duration of immunity. Unfortunately, it was not possible to follow the course of humoral immunity in a large number of animals because of the death of some and the use of others in the reinoculation experiments; however, sufficient data had been obtained to arrive at certain conclusions.

Table 7 summarizes the results of the neutralization tests on the sera, obtained at various intervals after virus inoculation, from the group of animals that circulated virus and developed antibodies.

Most of the animals developed detectable antibodies by the end of the second week following the infectious dose. A more detailed study of the data has

revealed that actually the majority of animals have shown their maximum antibody response at this time, as was revealed by the greater proportion of the higher survival ratios. However, a few animals have shown the maximum response only after 30 days. Once having developed antibody, one of two courses ensued: either the animal maintained its neutralizing antibody (as judged by the survival ratio), or the antibody level began to decrease. As can be seen in the table, this decrease in antibody began almost immediately in some of the animals, and as the interval from the time of inoculation increased, more and more of the sera were found to have normal survival ratios. No consistent relationship was observed between the rate of disappearance of the neutralizing antibody and the intensity of the earlier antibody response. Some animals whose sera gave high survival ratios, even in tests employing over 100 LD₅₀, were found to lose antibody at a very rapid rate, sometimes showing a decrease from a 6/6 survival ratio to 0/6 ratio within a few months. The sera of others, never having a high survival ratio, would maintain this level of neutralizing antibody

TABLE 7

Results of neutralization tests on sera obtained at various intervals after yellow fever infection of C. laniger

NO. OF ANIMALS TESTED	DAYS AFTER INFECTION DOSE	NEUTRALIZATION TEST SURVIVAL RATIOS*			PER CENT NEG.
		6/6, 5/6	4/6, 4/5, 3/6, 3/5, 3/4, 2/6	1/6, 0/6, 0/5	
35	12-24	19	13	3	9
40	29-51	14	20	6	15
25	60-228	9	9	7	28
7	301-389	2	1	4	57

* The figures indicate the number of animals whose sera gave the specified response.

for long periods of time. Sera of some animals maintained a high survival ratio for 389 days, the longest interval tested. No relationship was observed between the amount of antibody formed (as reflected in the survival ratio) or its duration, and the intensity of the infection as judged by the length of time virus circulated or the amount of virus in the circulation. However, once an animal lost detectable neutralizing antibody, this antibody was never observed to increase spontaneously. Also, sera obtained at various intervals after exposure to infection, from those animals that failed to circulate virus or to produce antibodies, remained consistently negative.

The challenge of previously inoculated woolly opossums. The reinoculation experiments offered an opportunity to investigate several questions that arose as a result of the initial experiments. There was a chance to prove that the antibody formation observed was in reality specific yellow fever antibody. Further, it could be determined whether those animals that had lost detectable neutralizing antibody were again susceptible to yellow fever. The results of the first susceptibility tests seemed to indicate that *C. laniger* could be divided into two groups as regards their susceptibility to yellow fever virus: one group

which was more or less uniformly susceptible, and the other which was resistant. It was possible to imagine a third group in which the susceptibility was dependent on the dose of virus inoculated. The experiments to be described have yielded information on each of these points.

Thirty-two opossums were inoculated subcutaneously with large doses of yellow fever virus at varying intervals after the first exposure. In all of the challenge experiments the Chichimene strain was used; the dose in most instances was between 10^4 and 10^5 LD₅₀. On one occasion, a small group was challenged with only 10^3 LD₅₀. Tests for circulating virus were performed according to the technique previously employed.

The animals comprised four distinct groups based on their first reaction to yellow fever:

Group I. Animals that, following the circulation of virus, had developed neutralizing antibodies which had persisted and were present at the time the challenge dose was administered.

Group II. Animals that had circulated virus and developed neutralizing antibodies but whose sera did not contain detectable humoral antibodies at the time of challenge.

Group III. Animals that had circulated virus but did not develop sufficient antibody to give a survival ratio greater than 1/6 in a neutralization test employing small doses of virus.

Group IV. Animals that failed to circulate virus following the first inoculation of yellow fever virus and showed no evidence of neutralizing antibody in post-inoculation serum samples.

The pertinent data obtained in these experiments is presented in table 8.

Animals included in group I had been inoculated between 51 and 389 days previously, 11 of the 15 having received the first dose of virus between 98 and 161 days before the second. None of these animals circulated virus following the second inoculation. All, naturally, had neutralizing antibody in the post-inoculation serum sample.

In group II, eight animals were tested and only one circulated virus. This opossum had circulated virus following inoculation of the Chichimene strain 389 days before the second dose. Neutralizing antibody had developed after the first virus inoculation, as evidenced by a 2/6 survival ratio in a neutralization test with 31 LD₅₀. This small amount of antibody was apparently lost rather rapidly, as a serum sample obtained 178 days after the first inoculation was negative, giving a 0/6 survival ratio with an average survival time of 4.7 days in a neutralization test employing 80 LD₅₀. Serum obtained from this animal 16 days after the second dose of virus gave a 1/6 survival ratio with an average survival time of 7.3 days which, although classified in the table as "negative" by the previously established criteria, may in reality indicate the presence of a small amount of antibody, especially since the preinoculation sample, tested at the same time, gave a 0/6 survival ratio with an average survival time of 5.3 days in a neutralization test employing 50 LD₅₀.

Group III was composed of two animals that had previously circulated virus

but whose postinoculation sera did not have sufficient neutralizing capacity to be classified as "immune" by the criteria discussed above. In each instance, however, the postinoculation serum samples gave slightly prolonged average survival times. The serum samples, though, obtained just prior to the second inoculation of virus were considered to be really "negative." One animal was challenged 49 days after the first dose, and the other, which circulated virus again, received the

TABLE 8

Results of the second inoculation of *C. laniger* with the Chichimene strain of yellow fever virus

NO. OF ANI- MALS	PREINOCU- LATION NEUTRALI- ZATION TEST RESULTS	NO. OF ANIMALS CIRCULATING VIRUS ON GIVEN DAYS AFTER INOCULATION*									NO. OF ANIMALS CIRCULA- TING VIRUS	POSTINOCULATION NEUTRALIZATION TEST RESULTS				
		2	3	4	5	6	7	8	9	10		No. of animals bled	Virus in circulation		No virus in circ- ulation	
													Pos.†	Neg.†	Pos.†	Neg.†
Group I†																
6	P	—	—	—	—	—	—	—	—	—	0	4			4	
9	P	0	—	—	—	—	0	0	0	0	0	8			8	
Group II																
8	N	—	—	1	—	—	—	—	—	—	1	8		1	7	
Group III																
2	N	0	1	—	—	—	0	0	0	0	1§	2	1		1	
Group IV																
4	N	—	3	2	2	—	1	—	—	—	3	3	3			
3	N	0	1	1	1	0	0	0	0	0	1¶	3	1		2	

* — signifies that the animals were bled but did not have detectable virus in the blood.
0 signifies that the animals were not bled. The figures indicate the number of animals that circulated virus on the given day after inoculation.

† The figures represent the number of animals whose sera gave the indicated response.

‡ See text for an explanation of the types of animals comprising the various groups.

§ This animal had circulated virus following the first inoculation 112 days previously.

|| The animal not circulating virus did not survive for the postinoculation bleeding.

¶ This animal was reinoculated 541 days later and again circulated virus.

second dose just 112 days following the first inoculation. Both animals developed neutralizing antibodies following the second dose. However, the animal that circulated virus lost the small amount of antibody produced, as a serum sample obtained 236 days after the second inoculation was again negative, giving a 0/6 survival ratio with an average survival time of 4.3 days in a neutralization test with only 32 LD₅₀. The other opossum, though not circulating virus, developed antibodies, and a serum sample obtained 103 days after the second dose still showed quite marked protection in the neutralization test.

Group IV, composed of animals that neither circulated nor developed detectable neutralizing antibodies following the first inoculation, reacted somewhat

unexpectedly to the second dose of virus. Seven animals were tested, and four of them circulated virus. Furthermore, two opossums that did not circulate virus developed neutralizing antibodies, which they had not done following the previous test.

It was noticed that the immune reaction of the animals comprising the Groups I, II, and III was somewhat stronger following the second inoculation than after the first infection. However, many of the animals that lost antibody following the first dose of virus showed the same tendency to lose antibody following the second.

It might be interesting to review in detail the history of one of the animals in Group IV, as many of the diversities encountered in *C. laniger* as a species in its reaction to yellow fever virus may be seen in this one example. This opossum was first inoculated with 1,000 LD₅₀ of the Chichimene strain and failed to react either by circulating virus or by producing neutralizing antibody. Two hundred and two days later a second dose of 13,333 LD₅₀ of the same strain was followed by the circulation of virus and the production of neutralizing antibody. The detectable antibody disappeared rather rapidly, and a sample of serum obtained 236 days after the second dose was negative, as was a sample obtained 305 days later (541 days after the second dose). At this time a third dose of 16,666 LD₅₀ of the Chichimene strain was inoculated, and virus was again demonstrated in the circulation.

A few of the animals that failed to circulate virus but did produce antibody following the first inoculation of virus have, also, been retested. This group has shown the same tendency to lose neutralizing antibody as have the other animals. None of these opossums circulated virus following the second dose, but all had strongly protective postinoculation sera.

DISCUSSION

On the basis of the responses obtained following the first dose of virus, it was possible to separate the experimental animals into three groups: (a) animals that circulated virus; (b) animals that developed antibody without detectable circulating virus; and (c) animals that neither circulated virus nor produced antibody. The group that circulated virus was obviously susceptible, but the status of the two latter groups was uncertain until the reinoculation experiments were completed.

Interpretation of the results obtained from the animals that did not circulate virus but developed neutralizing antibody was and has remained confused by two completely different sets of data, either of which would explain the findings provided the circumstances were different. First, it was demonstrated that virus could at times be isolated from infected animals by inoculation of the serum intracerebrally into baby white mice when the same serum failed to infect adult mice, indicating that the standard test for circulating virus was not always sufficiently sensitive. Second, studies of the neutralizing antibody of animals that had circulated virus showed that detectable antibody often disappeared from the circulation. Reinoculation of these animals resulted either in their again

circulating virus or producing neutralizing antibodies. Since the animals came from endemic yellow fever areas it has been impossible to differentiate those animals that circulated virus in non-detectable amounts from the wild-caught immunes that had lost humoral antibody. Theoretically, it makes no difference, as the immunes would be assumed to have been susceptible at an earlier date.

Unfortunately, it was possible to reinoculate only seven of the group of 18 animals that failed to react to the first virus inoculation either by circulating virus or by producing antibodies. However, following the larger dose, *all* of these animals reacted either by circulating virus or by producing antibodies. Since no selection of individuals was made it may be assumed that most of the 11 other animals, had they survived for the test, would have reacted in much the same manner.

The results of the reinoculation studies served to modify the first impression concerning the susceptibility of this species, an impression based on the first experiments with Chichimene virus. The distribution of animals circulating virus in relation to the dose of Chichimene inoculated was at first interpreted as indicating that susceptibility was an "all or none" phenomenon. However, the finding that all of the animals reacted to the inoculation of a larger dose of the same virus clearly demonstrated that the susceptibility of at least some of them was dependent on the dosage. The failure to obtain a distribution indicative of this in the beginning could be explained by two observed phenomena: first, the variation in susceptibility in relation to the dosage of virus extends over a broad range; and second, the number of animals resistant to even a small dose is not great.

The data obtained on the susceptibility of the woolly opossum to strains other than the Chichimene strain are not sufficient, especially when a possible dosage factor must be considered, to justify any opinion as to the significance of the small difference in infectivity observed among strains. Suffice it to say that all of the strains employed were found to be infectious for *C. laniger*.

The significance of the susceptibility studies from the epidemiological point of view is somewhat difficult to evaluate. The group of animals that was susceptible only to large doses would probably not be considered to be susceptible epidemiologically, as the probability of encountering such large doses in nature would seem, in the light of the information available at present (12), to be small indeed. Considering the data presented by Bates (3), the susceptibility of *C. laniger* is very similar to that of *Metachirus nudicaudatus*. Recently, Bates and Roca (13) have been able, with considerable irregularity, to infect *Haemagogus spegazzinii* falco on *Metachirus nudicaudatus*. These authors failed, however, to infect the same species of mosquitoes on *C. laniger*, but fewer attempts were made. In view of the low and fluctuating virus titers in the woolly opossums, it would seem reasonable to suggest that attempts to infect mosquitoes might be more successful if the mosquitoes were fed in small groups at intervals during the period of virus circulation instead of all at one time or in two groups.

Normal sera from woolly opossums behaved very much like normal human sera in the intracerebral neutralization test. The non-specific viricidal activity reported in the sera of other species of marsupials was not encountered in sera of

C. laniger (1, 3). Studies of the neutralizing antibody response of infected opossums have revealed certain differences from responses usually encountered in primates. Occasionally, it was not possible to detect with certainty neutralizing antibody formation in animals that had circulated virus. This failure of some individuals to produce significant amounts of antibody following infection with viscerotropic yellow fever virus, although without explanation, recalls the failure of a significant number of humans to produce detectable antibodies after vaccination with the 17D strain of yellow fever virus. Also, a number of animals that were found to be immune to reinoculation of yellow fever virus, apparently had lost their once detectable antibodies. However, false serum reactions of the type mentioned by Bates (3) were not encountered; no animal with a positive neutralization test just before inoculation circulated virus. It is possible that one of the more sensitive tests recently described (14, 15) may prove effective in detecting the small amount of antibody present in some of these sera.

From the epidemiological viewpoint it can be seen that neutralizing antibody in *C. laniger*, as detected by the intracerebral neutralization test, is not a reliable indicator of past infection with yellow fever inasmuch as the sera of many immune animals fail to neutralize the virus. The per cent of immunes encountered in a given locality, on the basis of the foregoing data, would be expected to vary both with the time since exposure and the number of animals infected.

SUMMARY

It was found that 58 per cent of *C. laniger* injected with 1,000 LD₅₀ of yellow fever virus circulated virus following the inoculation. The virus content of the serum of the infected animals was found to be small in most instances, the majority of titers being less than 1/100. The susceptibility of some of the animals was shown to depend on the amount of virus inoculated.

Over 90 per cent of the animals circulating virus developed neutralizing antibodies. Frequently these antibodies were present in small amounts, and their detection was possible only in neutralization tests employing small test doses. However, since no evidence of non-specific viricidal activity was found in normal woolly opossum sera, it was considered that a survival ratio of 2/6 or greater indicated the presence in the serum of neutralizing antibodies. Studies on the persistence of neutralizing antibodies in the serum revealed that a significant number of animals lost these antibodies during the course of a year after inoculation.

Reinoculation of previously infected animals with large doses of yellow fever virus showed that occasionally animals would completely lose their immunity and circulate virus following the second dose of virus. In some cases it was found that although the animal had lost detectable humoral immunity, no virus could be isolated from the blood, which demonstrated that the intracerebral neutralization test result is not always a reliable indication of the immune status of the animal. Animals with neutralizing antibody at the time of inoculation in no instance circulated virus, thus establishing the specificity of the neutralization test.

The epidemiological implications of the data are discussed.

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REDUCTION OF ANOPHELES DENSITY EFFECTED BY THE PRE-SEASON SPRAYING OF BUILDING INTERIORS WITH DDT IN KEROSENE, AT CASTEL VOLTURNO, ITALY, IN 1944-1945 AND IN THE TIBER DELTA IN 1945¹

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Received for publication Jan. 13, 1946

INTRODUCTION

In the spring of 1944 the Surgeon of the Mediterranean Theater of Operations of the United States Army (MTOUSA) requested that the members of The Rockefeller Foundation Health Commission who were working in Italy undertake studies of the use of DDT for the control of house-infesting anopheles of the Italian malaria-vector species, *Anopheles labranchiae labranchiae*. At the time, the area facing the Allied Armies had been extensively flooded and mined by the retreating German Army so that the customary application of larvicides for malaria control was hazardous if not impossible. Later, the Allies Control Commission (ACC) for Italy issued a formal invitation to the members of the Health Commission to organize a Malaria Control Demonstration Unit under the auspices of its Public Health Sub-Commission.

One object of the investigation was to determine the effect upon anopheles of treating the interiors of all buildings with DDT in the absence of other measures for their control. The area chosen for the initial studies was a part of the Bonifica di Castel Volturno extending along the southwestern coast of Italy between the Volturno River and the Lago di Patria. Later, similar but more extensive investigations were carried out in the Tiber Delta. In this paper the activities in the Castel Volturno region in 1944 and 1945 will be summarized briefly, and the work in the Tiber Delta will be discussed at greater length.

CASTEL VOLTURNO, 1944

A permanent river, the Regi Lagni, formed by the junction of the Aprano and Lagni streams flows from east to west, bisecting the 1944 experimental area at Castel Volturno in such a way that the southern of the two sections was approximately twice the size of the northern one. Prior to the outbreak of the war this previously swampy area had been drained and partly settled. In September 1943 the Germans destroyed the pumping stations, thus flooding the region along the Lagni, and particularly that south of the river.

¹ The studies and observations on which this report is based were made at the request of the Surgeon's Office of the Mediterranean Theater of Operations of the United States Army by members of The Rockefeller Foundation Health Commission functioning as the Malaria Control Demonstration Unit of the Malaria Section of the Public Health Sub-Commission, Allied Control Commission for Italy. Essential contributions were made by the Istituto Superiore di Sanità, Rome, by the Health Department of the Commune of Rome and by the malaria control groups of the British and United States Armies.

The test area was arbitrarily divided into six sections, as shown in the accompanying map (Fig. 1). Sections A and B lay between the Lagni and Volturno rivers, C and D were south of the Lagni and E and F lay between C and D and the Lago di Patria. It was decided that the interiors of all buildings of sections B and D should be sprayed with a 5 per cent solution of DDT in kerosene, that those of sections E and F should be dusted with 10 per cent DDT in pyrophyllite and that the buildings in sections A and C should remain untreated for observation purposes.

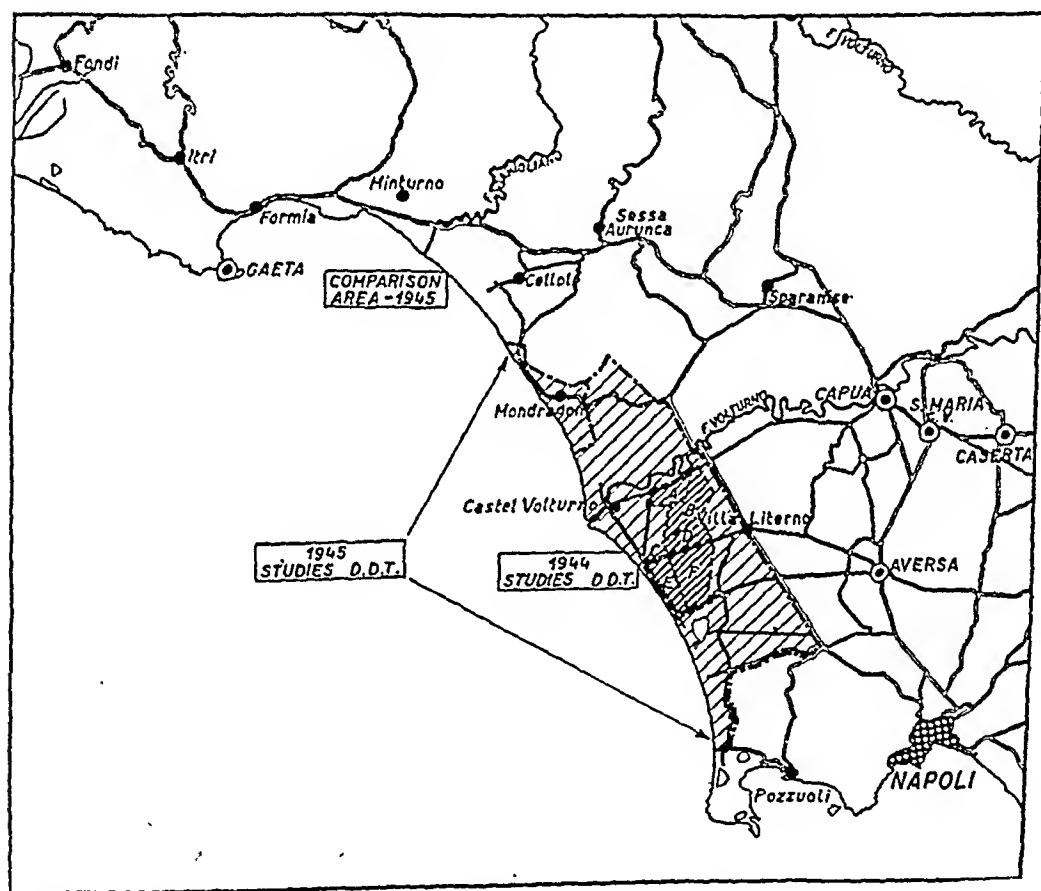


FIG. 1. CASTEL VOLTURNO AREAS COVERED IN RESIDUAL DDT STUDIES, ITALY, 1944 AND 1945

During the week beginning May 17, DDT spray or dust was applied to the walls and ceilings of all rooms, i. e., bedroom, kitchen, stairway, stair well, stable, pigsty, chicken-run, toilet, cartshed and oven, of all buildings in sections B, D, E and F. The DDT solution was applied with an ordinary hand-operated knapsack sprayer with a disk-type nozzle giving a conical spray. Approximately a quart of liquid was used per 1,000 square feet, giving an estimated dosage of 60 milligrams of DDT per square foot of sprayed surface.

The DDT powder was applied with a plunger-type hand dust-gun, the Hudson "Admiral." More powder adhered to the wall when the gun was held nearly

parallel to it with the stream of powder directed toward the ceiling. A deposit of not more than 20 milligrams per square foot was thus obtained.

The buildings of sections E and F were redusted during the week beginning July 9; and between August 7 and 27 one team of four men sprayed the buildings on all the 216 farms in the four sections, applying approximately 80 milligrams per square foot of treated surface.

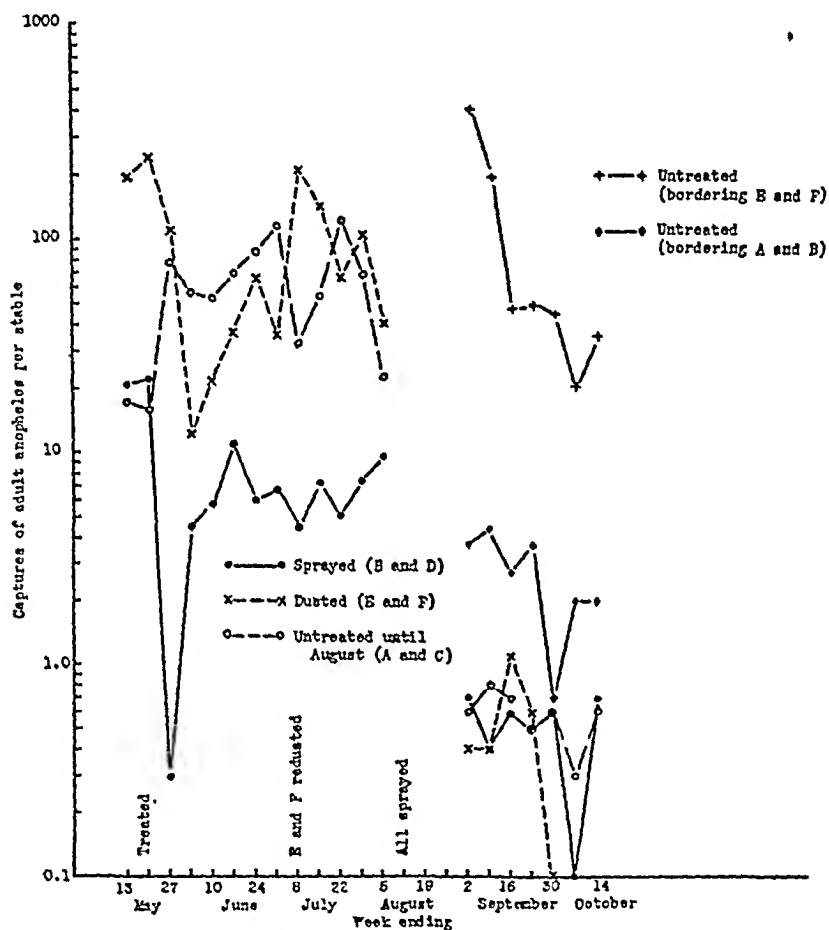


FIG. 2. CASTEL VOLTURNO, 1944: ANOPHELES CAPTURED WEEKLY IN TREATED AND UNTREATED SECTIONS

Certain premises in each section were selected for weekly captures of adult anopheles. Two inspections were made before the insecticide was applied, and subsequent captures were made routinely through July 8 and from August 10 through October 14. Captures were limited to a designated strip of bedroom ceiling 1 meter wide and 4.3 meters long and to an area of 1 square meter at each of the four corners of the stable roof.

Figure 2 shows the trend of the weekly captures in stables in the four treated and two untreated sections of the area. Since captures prior to treatment in sections E and F were 10 times as high as those in sections B and D, the superior effect of the 5 per cent DDT in kerosene was not clearly demonstrated until

after all buildings had been sprayed in August. That even the powder is effective is shown by a comparison of the trends in anopheles density in sections E and F with those in the untreated sections A and C.

When captures were resumed in September density in all sprayed sections had dropped to less than one anopheles per premises. For comparison, captures from two districts outside the study area are shown in Figure 2. In both the postseasonal decrease in anopheles density had set in but the levels were well above those in the treated sections.

Surveys made in June, August and October to determine the species of anopheles present revealed *A. labranchiae*, *A. sacharovi*, *A. melanoon* and *A. messeae*. While *labranchiae* was dominant early in the season, *sacharovi* became more prevalent later.

Spleen and parasite surveys were made of all school children, 6-14 years of age, in the study area prior to the treatment of the premises in May and later in August and October. Thick and thin blood smears were examined for parasites, and spleen enlargement was recorded by Schüffner's method. During this period spleen rates varied from 43 to 39 per cent, while parasite rates dropped from 21 to 8 per cent.

CASTEL VOLTURNO, 1945

A study of residual effects of DDT at Castel Volturno in 1945 was conducted by Malaria Control Units, MTOUSA, with the Malaria Control Demonstration Unit retaining responsibility only for the parasite and spleen surveys. A short résumé of a report on this study by Aitken (1) is given below.

The 1944 test area was enlarged to comprise a coastal strip approximately five miles wide, extending from the Mondragone hills south to the village of Qualiano, thence following the hills in a southwesterly direction to Mt. Cuma. The zone thus defined included approximately 95 square miles (Fig. 1). Approximately 85 milligrams of DDT were applied per square foot.

Routine captures were made weekly from December 22, 1944, through September 1945 in selected treated premises over a somewhat larger surface than was covered by captures in 1944. No anopheles were captured in December and January in houses sprayed in August, while hibernating mosquitoes in moderate numbers were found in untreated houses.

In April 1945 captures were inaugurated in untreated premises in the Bonifica di Sessa, a thinly populated section south of the Garigliano River, and in the town of Minturno where the surrounding terrain was flooded. Because of the swarms of anopheles present, inspections were made semimonthly and counts were based on estimates when there were presumably more than 1,000 mosquitoes on the examined surface. The following record of captures per square yard in treated and untreated sections for the months of April through September speaks for itself:

AREA	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER
Treated.....	0.001	0.003	0.05	0.13	0.04	0.06
Untreated.....	9.87	41.84*	167.04*	118.50*	185.54*	185.54*

* Based on estimates of over 1,000 mosquitoes.

In the area treated in 1944, spleen and parasite surveys were again made in January, May and August of 1945, and the trend of the rates obtained in the six surveys is shown in Figure 3. For comparison, the 1945 May and August rates for the untreated Sessa area are shown also. Although the spleen rates in the test area did not fall significantly during the period of observation, they did not rise, and the Sessa rates were definitely at a higher level. On the other hand, parasite rates in the treated zone dropped significantly from 21 per cent to 1.4 per cent during the interval, while the Sessa rate rose from 18 per cent in May to 41 per cent in August, when transmission was at its height.

The 1945 studies in Castel Volturno indicated that a single pre-season application of 85 milligrams per square foot of DDT in kerosene to walls and ceilings in

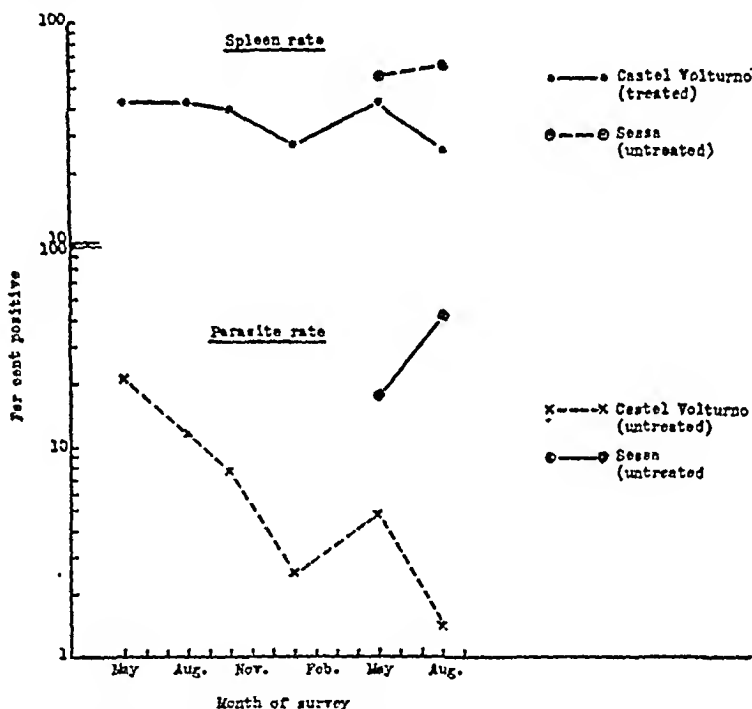


FIG. 3. CASTEL VOLTURNO, 1944-1945: TREND OF SPLEEN AND PARASITE RATES AMONG CHILDREN FROM 6-14 YEARS OF AGE IN TREATED AND UNTREATED AREAS

rooms of all buildings on all premises will greatly reduce the density of anopheles in their habitual resting places for the entire breeding season. While spleen rates remained high during the period of observation parasite rates dropped sharply at a time when both spleen and parasite rates in the untreated area were rising.

THE TIBER DELTA, 1945

Plan and sponsorship of the project.—During the summer of 1944 the Malaria Control Demonstration Unit also collaborated in an Allied project in the Tiber Delta by applying a 5 per cent DDT solution in kerosene to the interiors of buildings occupied by troops in Ostia Lido and to all farm buildings in the Isola Sacra formed by two branches of the Tiber River at its mouth. The efficacy of DDT in reducing anopheles density was further demonstrated here.

The 1945 investigation was designed to determine the effect of a single heavy application of a DDT spray in all human habitations and animal shelters in the early spring. The advantages of the Tiber Delta as a site for this study were: easy accessibility from Rome, the variety of agricultural development, the high malaria potential, the protection afforded against infiltration of anopheles from outside, together with the willingness of both civil and military authorities to surrender to the Malaria Control Demonstration Unit all responsibility for control of anopheles in the Delta during the 1945 season.

Plans were submitted to, and approved by Brigadier G. Parkinson, Chief of the Public Health Sub-Commission of the Allied Commission for Italy, and Colonel W. S. Stone, Chief of the Preventive Medicine Section, Office of the Theater Surgeon, MTOUSA. The Rome Area Allied Command Malaria Control Group under the command of Colonel T. D. Inch (British) was kept informed of all details of the project.

Excellent cooperation was received from the 10th Malaria Field Unit (Captain Johnson, British) and Malaria Control Unit No. 133 (Captain Mood, American) that were responsible for protecting Allied forces in the Rome area. The Italian Hygiene and Health Department for the Commune of Rome, through Professor Cramarossa, Public Health Officer, collaborated by suspending all antimalaria activity in the test area during the season, and by contributing to the project the services of employees usually engaged in such work. Finally, belief in the ultimate success of the project led Professor Missiroli, of the Istituto, di Sanità Superiore, to forego all antimalaria measures in Maccarese, where he had carried on control work for many years and where in 1944 he had made a careful test of the effect of suppressive atabrine treatment.

The test area.—The Tiber Delta, as here designated, is an irregularly shaped district of some 216 square kilometers (120 square miles) bordering the Tyrrhenian Sea about 20 kilometers (12.4 miles) southwest of Rome. It extends from Palo on the north 37.5 kilometers (23.3 miles) to Tor Paterno on the south, with a maximum width of about 18 kilometers (11.2 miles) at the point where the river zigzags across the plain. The terrain is flat, averaging 2 to 3 meters above the level of the sea, and contains some depressions extending below sea level.

The political districts included in the test area were Acilia, Ostia Antica and Ostia Lido, Ponte Galera, Fiumicino, Maccarese and Palidoro, all belonging to the Commune of Rome. For administrative purposes the test area was subdivided into four sections as shown in Figure 4, Acilia (E), Ostia (D), Fiumicino (C) and Maccarese (B).

For the conclusive studies planned for 1945 it was important that the test area be so large that infiltration of anopheles at the periphery would not destroy the effect of treatment at the center, and that the district be protected from invasion from without. The Tyrrhenian Sea provides a barrier on the south and west. To the north low rolling hills form a divide, which for about 3 kilometers is dry and free from permanent water courses. The north-northeast boundary is protected by the Roman Hills to a point about $1\frac{1}{2}$ kilometers north of Ponte Galera. Several streams descend from these hills, but during the

summer of 1945 these were dry. East of the test area and north of the Tiber, buildings were sprayed at the request of the British 10th Malaria Field Unit, while the area south of the Tiber was similarly treated by the American Malaria Control Unit No. 133. Thus the possibility of infiltration of anopheles from contiguous areas was reduced to a minimum. The periphery to the south was sparsely populated, and no evidence of infiltration there was recorded during the season.

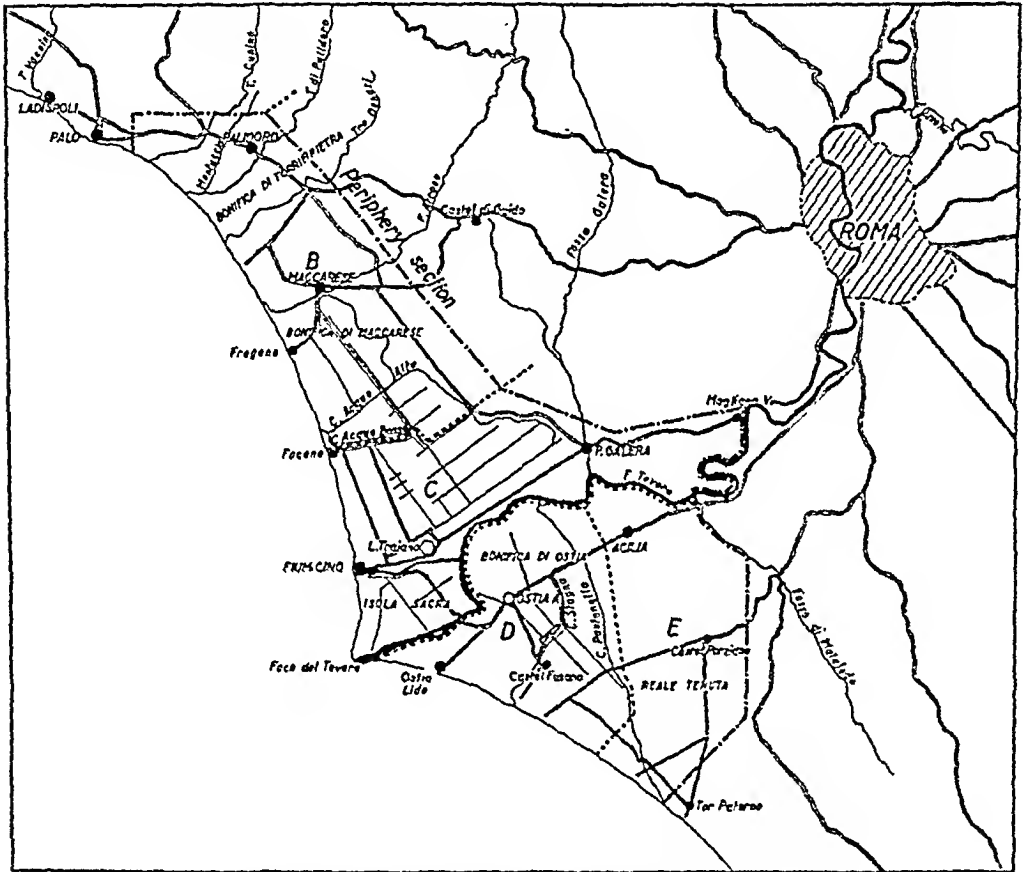


FIG. 4. TIBER DELTA AND COASTAL PLAIN TEST AREA

In recent times prior to 1889, the Tiber Delta was virtually uninhabited. When in flood the river overflowed into the wide marshes called "Stagno di Maccarese" and "Stagno della Pagliete." Even the docks of the ancient Porto di Traiano, situated at some distance from the sea, were frequently inundated. The sparse population of shepherds lived in the medieval castles of Ostia Antica, Fusano, Porziano, Maccarese and Palidoro and on occasional farms. Brush and forest covered the remainder of the district. A small fishing village, Fiumicino, was situated near the mouth of the navigable canal of the Tiber.

Reclamation of the Delta was begun by the Italian Government between 1885 and 1890, but it was not until after World War I that extensive agricultural

drainage and colonization were seriously undertaken. Thus, within a few years, brush, forest and swamps were replaced by farms which supported large herds of cattle, extensive vineyards and market gardens. Modern villages made their appearance. Excellent highways now connect every farm with its market center. Much of the Delta is irrigated as well as drained. The southern and southwestern portion, however, has never been reclaimed. Known as the "Tenuta di Castel Porziano" it is maintained as a royal hunting reserve for the conservation of wild life.

From reliable Italian sources it has been learned that the Germans gave orders that the Maccarese pumping station should stop working on October 9, 1943, but that the pump which lifted water from the Tiber to the distributing canals of the irrigation system should continue to operate, thus causing extensive flooding of the Maccarese plain. On October 19 the drainage pumps on the Isola Sacra ceased to function, by German order, while the tide gates on the outlet canal were permanently opened. Two of the pumps at Ostia Antica were destroyed and the main effluent canal to the sea was blocked in three places. As the coastal area of the Delta is at or slightly below sea level, these steps led to extensive flooding during the rainy season of 1943-1944.

Malaria in the Delta.—Data on malaria morbidity are available for this area since 1927 at the Hygiene and Health Department of the Commune of Rome. A case is recorded as primary if the patient has not formerly been treated by the health unit, and as secondary if he is listed in the records as having had prior attacks. Presumably the diagnosis is based on the finding of parasites in a blood smear. Since it is the morbidity of the period just prior to World War II which is of special interest for the purposes of this report, annual rates for the period 1936-1945 are shown in Figure 5 for six of the sanitary units lying wholly or in part within the test area. Data for the town of Ostia Lido are not included.

In 1936 malaria morbidity in the units of Fiumicino, Ostia Antica and Ponte Galera was less than 1 per cent while that in the units of Acilia, Maccarese and Palidoro was between 4 and 5 per cent. In Fiumicino and Ostia Antica the rates pursued a parallel course, rising to a minor peak in 1939 and then sharply to a major peak in 1944 following the inundation caused by the destruction of the drainage equipment. In Acilia and Ponte Galera there were increases in 1939 and 1944 separated by a period of lower incidence in 1940-1943. The increase in morbidity in 1939 has been attributed to the introduction of non-immune laborers for the construction of a large hydroplane base on the plain of Magliana. This work was discontinued when Italy entered the war. These areas, located on higher ground were not seriously affected by the inundation of 1943-1944.

Morbidity rates in the Maccarese and Palidoro units pursued similar courses. Incidence in Palidoro dropped continuously from 1936 through 1942 and then rose, while in Maccarese there was a transient rise in 1939 and 1940, a drop to a minimum in 1941 followed by a sharp rise in 1942. Although the Maccarese area suffered extensively from the inundation in 1943-1944, a program of sup-

pressive treatment with atabrine¹ was instituted by Professor Missiroli in the summer of 1944. Palidoro was not affected by the inundation, but maintenance of its drainage system lapsed. Atabrine was administered here also in 1944.

In May 1945 a survey of 2,268 school children in the coastal plain was made by the same group that conducted the surveys in Castel Volturno in 1944 and 1945 to obtain spleen and parasite indices of malaria infection. The results

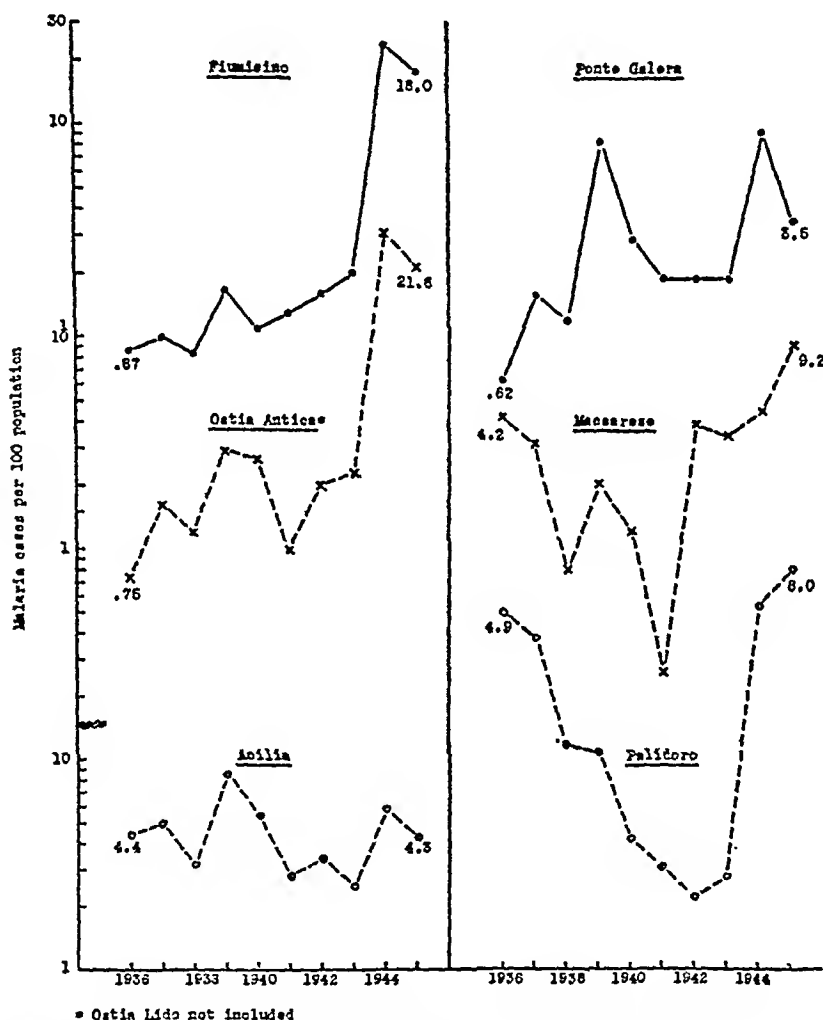


FIG. 5. TIBER DELTA: TREND OF MALARIA MORBIDITY BY SANITARY UNIT, FOR THE YEARS 1936-1945

are given in Table 1. The rates vary considerably. The proportion of children with enlarged spleens ranged from 12 per cent in Maccarese to 67 per cent in Lingua D'Oca, while the spleen rate for all children was 18 per cent. Parasite rates were lower, ranging from 1 per cent in several towns to 26 per cent in Porto Vecchio, with an average for the total group of 4 per cent.

The anopheles vector. *Anopheles labranchiae labranchiae* is the most dangerous

of the Italian malaria vectors. Missiroli (2) has shown that after the inundation of the Delta area in 1943-1944 the percentage of labbranchiae among anopheles caught at three stations rose from 45, 29 and 48 to 96, 100 and 97 respectively. When breeding freely it is found in enormous numbers resting on walls and ceilings of buildings occupied either by man or animals. The choice of resting place of this species renders it particularly vulnerable to the residual effect of DDT.

The records of weekly captures of adult anopheles in 10 stations by the unit of Ostia Antica were obtained from the Hygiene and Health Department of the Commune of Rome, and monthly averages were computed from the data for 1939-1942. The trend of these averages for the months of May through

TABLE 1

Results of the spleen and parasite survey among school children of the coastal plain of Rome, May 1945

TOWN	NUMBER EXAMINED	NUMBER WITH ENLARGED SPLEENS	SPLEEN RATE PER CENT	NUMBER WITH PARASITES	PARASITE RATE PER CENT
Maccarese.....	600	72	12	30	5
Palidoro.....	218	37	17	9	4
Ponte Galera.....	108	16	15	1	1
Porto Vecchio.....	42	22	52	11	26
Isola Sacra.....	98	32	33	6	6
Lingua D'Oca.....	21	14	67	3	14
Porto Ponte Galera.....	53	8	15	3	6
Fiumicino.....	300	89	30	23	8
Ostia Antica.....	276	48	17	4	1
Castel Porsiano.....	26	5	19	1	4
Infernetto.....	21	5	24	1	5
Acilia.....	212	30	14	3	1
Ostia Lido.....	293	36	12	4	1
Total.....	2,268	414	18	99	4

November is shown in Figure 6. It should be noted that these densities pertain to years when there was active control of anopheles breeding through cleaning of ditches and the application of Paris green. A typical uncleaned ditch in 1945 is shown in Figure 7. For comparison with the earlier densities, captures from June through November in 1945 following treatment in the area south of the Tiber are shown in Figure 6. The difference in level is conspicuous.

Organization and equipment of field personnel.—A chief inspector was assigned to each of the four sections to be responsible for all equipment allocated to his section, to supervise the work and to keep records of the amount of insecticide issued and applied and of the number of buildings and rooms treated. The date of treatment was permanently stencilled on each group of buildings when the spraying was completed.

Each spraying gang consisted of a foreman and seven laborers traveling in a truck, the driver of which was also a member of the squad. The foreman carried a map of the district and was expected to keep careful records of the premises sprayed.

Raincoats, gloves, goggles and helmets were provided for each workman. Soap for daily baths was issued weekly together with laundry soap for washing the work garments. The use of gloves was discontinued immediately, and the

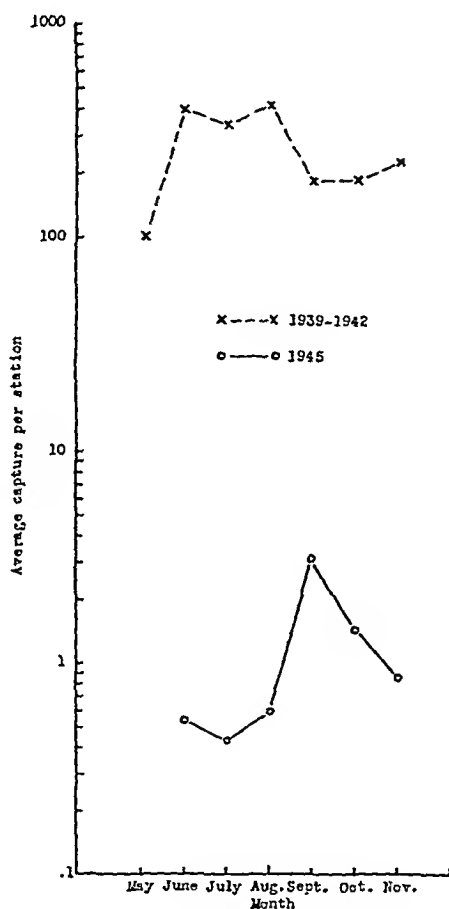


FIG. 6. TIBER DELTA: THE MONTHLY TREND (MAY-NOVEMBER) OF AVERAGE CAPTURES OF ADULT ANOPHELES IN OSTIA ANTICA, 1939-1942 AND 1945

goggles were discarded soon afterward, since they quickly became covered with a film of kerosene that interfered with vision. The men preferred their own hats or caps to the helmets. At no time during the spraying operations or later were there any complaints of ill effects of DDT, although some cases of dermatitis occurred which usually disappeared after a few days. These were attributed to the effect of kerosene rather than to that of DDT.

The insecticide was applied with a standard farm-type knapsack continuous

sprayer, except that the usual nozzle plate giving a cone-shaped spray was replaced by a disk furnishing a flat, fan-shaped spray. The standard delivery arm of 24 inches was too short for most of the work to be done. Two of these could be joined for spraying the lower part of the walls, but longer arms were devised for spraying the upper portions of high walls and ceilings, by inserting a light-weight pipe of $\frac{5}{16}$ -inch diameter through bamboo poles, 2, 3, 4 or 5 meters



FIG. 7. Use of modified 4.5 Liter sprayer. (From *Journal of Tropical Medicine and Hygiene*, 1954, 57, 101-102.)

longer, with the original handle. But the longer arms were fitted to the sprayer. A disk was used that of course took a little longer to fill, but it was worth it. A light-weight pipe was inserted in the wall and poles were used to hold the sprayer in place. The person applying the insecticide was on the ground.

A 2.5 liter sprayer was used in the 1954-55 season, and a 4.5 liter sprayer was used in the 1955-56 season. The 4.5 liter sprayer was used in the 1956-57 season.

rivers, drainage and irrigation canals, pumping stations, highways, bridges, and the location of every group of buildings. While the map was in preparation all premises were visited and the number and location of buildings were verified.

Preparation and application of the insecticide.—It was found that the kerosene available would readily dissolve considerably more than 5 per cent by weight of DDT, and since a heavy dose was desired, it was decided to use 6.5 per cent by weight in the solution. The insecticide was prepared at headquarters in Rome. After filtration, 205 liters of kerosene were poured into a clean 55-gallon drum into which 11.4 kilograms (25 pounds) of 100 per cent DDT had previously been dumped. The drum was then placed on a platform in the sun and agitated by rolling it frequently back and forth for a period of at least three days. In this manner 283 drums of solution were prepared containing 3,209 kilograms (7075 pounds) of DDT and 58,015 liters of kerosene. The solution was filtered when transferred to the sprayers since even fine particles in the liquid easily clogged the small orifice of the nozzle. Of the 283 drums of insecticide prepared, the contents of 275 were used in the initial spraying of buildings in the area.

Since an objective of the Tiber Delta study was to obtain information on the maximum effect of an application of DDT under Italian conditions, it was decided to use a dose of 200 milligrams of DDT per square foot of surface. The modern section of the town of Acilia had 226 identical two-family houses which served as a training base for field personnel. Each worker was trained to use approximately 11.5 liters of insecticide to cover the 275 square meters of surface to be sprayed in the interior of each of these houses.

The knapsack sprayer was operated by one man when a short delivery arm could be used, but when a longer arm was required two men were needed. The hose connecting the reservoir with the pole was lengthened to 2½ meters for two-man operation. Where large buildings with high ceilings or unceiled rooms were encountered, one group of operators, using sprayers with long delivery arms, treated the ceilings, another group equipped with sprayers having shorter arms, treated the walls just below the ceilings while a third group (or often a single workman) sprayed the lower part of the walls to within 1½ meters above the floor. The practice of not spraying the floor was adopted because of the impression that *A. labranchiae* did not rest low on the walls. It was, however, sometimes found resting in this zone during the summer.

The upper floors of houses were treated first. Everything in position against walls, with the exception of oil paintings, was sprayed—mirrors, wall hangings, pictures, window draperies, bed coverings, clothing, etc. Wardrobes were not sprayed inside. No special effort was made to spray behind pictures or furniture placed close to the walls or behind goods so placed in storerooms. Careful attention was always given to spraying electric light fixtures, ropes, wires or other hanging objects on which insects might rest. The housewife was advised to cover foodstuffs. Stables and outbuildings were sprayed without removal of animals.

Summary of spraying activities.—The time required for treating all the premises in each section is given below:

SECTION	DATE TREATMENT		SQUAD DAYS REQUIRED
	Began	Ended	
E—Acilia	March 2	March 30	28
D—Ostia	February 27	March 23	24
Ostia Lido	March 24	April 25	32
C—Fiumicino	March 7	April 27	51
B—Maccarese	April 4	June 15	41

In Table 2 is recorded the number and type of structures and of rooms sprayed in each section, together with the number of man-days expended on the job

TABLE 2

Tiber Delta, 1946: Man-hours expended, with the number and type of buildings and rooms sprayed, by section

SECTION	MAN-DAYS (STRAYERS ONLY)	NUMBER AND TYPE OF STRUCTURES SPRAYED							NUMBER AND TYPE OF ROOMS SPRAYED						
		Houses with animals	Houses without animals	Stables	Pigpens	Chicken-pens	Other	Total	Bedrooms	Other house rooms	Stables	Pigpens	Chicken-pens	Total	
E—Acilia.....	189	311	52	19	199	403	189	1173	1352	2997	97	297	1231	5,974	
D—Ostia.....	176	141	80	30	156	248	283	938	880	2186	130	292	781	4,269	
Ostia Lido.....	389	339	10	3	4	10	113	479	2414	16,888	2	4	177	19,485	
C—Fiumicino.....	359	634	147	29	7	4	821	1642	2464	5949	357	436	488	9,694	
B—Maccarese.....	507	528	104	59	82	91	773	1637	2672	5096	369	826	1566	10,529	
Total.....	1620	1953	393	140	448	756	2179	5869	9782	33,116	955	1855	4243	49,951	

Structures sprayed in addition to dwellings included all those which might serve as sources of blood meals to anopheles. In all, 5,869 buildings were sprayed requiring 1,620 man-days, a ratio of 3.6 buildings per man-day. There were 49,951 rooms of various types treated, a ratio of 30.8 per man-day.

Data pertaining to urban as distinct from rural portions of each section have been studied. The area treated covered 286 square kilometers of 110 square miles and housed 35,293 persons according to prewar census figures. From these data, ratios of persons per room and grams of DDT issued per room and per person have been computed for each subdivision of the area. These ratios vary within wide limits according to the type and size of building and to the number and kind of livestock sheltered. For the area as a whole there was less than one person per room (0.71); 63 grams of DDT were issued per room sprayed and 89 grams per person.

Postspraying capture of anopheles, larvae and adults.—As soon as a section

had been completely sprayed its personnel was reorganized and routine inspections for larvae and adult anopheles were inaugurated. The section was divided into small zones, each of which could be covered once a week by the inspector on a bicycle. Two men were assigned to each zone, one to look for larvae, the other for adults.

The procedure for adult captures in the Tiber Delta was unlike that in Castel Volturno in that inspections were made in all buildings on nearly all of the premises in the rural areas, so the counts should represent a large proportion of the anopheles present. Table 3 contains a record of adult captures from the week ending May 27 through that ending November 25, but the search for adult anopheles was continued on a reduced scale until February 20, 1946. Sections E (Acilia) and D (Ostia) south of the Tiber were combined for inspection purposes.

Larvae were captured at selected sites and the number taken in 20 dips with a net dipper was considered the count for the station. Table 4 contains the data for captures of larvae.

The trend of the weekly captures of adults for each of the three sections is shown in Figure 8. Such captures rarely exceeded one anopheles per premises and were frequently less than one in 10 premises. Density in Fiumicino (C) rose abruptly to a peak of 2.1 per premises during the week ending June 24 and then dropped, while a similar rise—not so precipitous—occurred in the Acilia-Ostia sections (E and D). Something like a delayed seasonal rise occurred in each section in September. Although density dropped to zero in Fiumicino during the week ending October 28, a minor rise at this time was observed in anopheles captured in the other sections.

All mosquitoes found were captured, and record was made of the number of anophelines and of culicines taken. Only 2,978 culicines were captured in the 28 weeks between May 14 and November 25, during which 10,544 inspections of premises were made. This gives an average of only 0.28 per premises inspected.

In Figure 9 weekly captures of adult anopheles in the test area are compared with those of selected peripheral stations in areas where breeding conditions were less favorable. While captures of adults attained a level of one per premises only once during the season in the treated sections, those in the peripheral stations ranged from approximately 4.5 to 25. Captures of larvae in the treated area attained a level of 2 per station the week ending July 1, dropped thereafter over a period of seven weeks, rose to a level of over 1 per station in August and September and then dropped sharply.

When adult anopheline density in the section of Fiumicino rose to two per premises the week ending June 24, the terrain and buildings at the site of the heaviest capture near Lago di Traiano were searched and a new pigsty, built after the other buildings had been sprayed, was found with some 300 mosquitoes resting on the walls and roof. Once sprayed it was never again found infested. Anopheles were also found in a large stable which had obviously not been properly sprayed. After re-treatment only one anopheles was subsequently found. Two

TABLE 3

Tiber Delta, 1945: Weekly captures of adult anopheles according to section

WEEK ENDING	PREMISES		ANOPHELES		PREMISES		ANOPHELES	
	Examined	Positive	Captured	Per premises	Examined	Positive	Captured	Per premises
	Sections E and D, Acilia and Ostia				All sections			
May 27.....	101	2	3	.030	101	2	3	.030
June 3.....	84	2	7	.083	84	2	7	.083
10.....	116	4	8	.069	263	9	19	.072
17.....	137	10	32	.23	323	21	154	.48
24.....	122	7	17	.14	307	32	405	1.32
July 1.....	110	4	13	.12	269	10	34	.13
8.....	122	4	8	.066	260	11	17	.065
15.....	141	9	18	.13	448	26	71	.16
22.....	152	9	20	.13	444	31	81	.18
29.....	148	4	8	.054	400	15	29	.073
Aug. 5.....	127	4	9	.071	398	22	60	.15
12.....	152	8	14	.092	476	30	97	.20
19.....	141	4	11	.078	440	26	203	.46
26.....	142	8	44	.31	473	34	143	.30
Sept. 2.....	161	8	8	.050	460	51	315	.68
9.....	138	7	13	.094	437	51	241	.55
16.....	147	10	59	.40	481	48	293	.61
23.....	147	11	171	1.16	462	48	352	.76
30.....	153	9	177	1.16	477	32	248	.52
Oct. 7.....	144	5	19	.13	461	18	39	.085
14.....	139	4	37	.27	462	13	57	.12
21.....	139	10	49	.35	463	29	91	.20
28.....	141	9	67	.48	452	25	116	.26
Nov. 4.....	110	8	35	.32	412	17	61	.15
11.....	141	18	31	.22	434	24	58	.13
18.....	140	13	37	.26	450	16	53	.12
25.....	139	10	27	.19	407	11	32	.079
	Section C, Fiumicino				Section B, Maccarese			
June 10.....	147	5	11	.075	—	—	—	—
17.....	186	11	122	.66	—	—	—	—
24.....	185	25	388	2.10	—	—	—	—
July 1.....	159	6	21	.13	—	—	—	—
8.....	138	7	9	.065	—	—	—	—
15.....	161	6	17	.11	146	11	36	.25
22.....	172	7	20	.12	120	15	41	.34
29.....	166	8	17	.10	86	3	4	.047
Aug. 5.....	133	8	24	.18	138	10	27	.20
12.....	177	6	30	.17	147	16	53	.36
19.....	152	7	13	.086	147	15	179	1.22
26.....	181	13	37	.20	150	13	62	.41
Sept. 2.....	158	20	130	.82	141	23	177	1.26

TABLE 3—*Concluded*

WEEK ENDING	PREMISES		ANOPHELES		PREMISES		ANOPHELES	
	Examined	Positive	Captured	Per premises	Examined	Positive	Captured	Per premises
	Section C, Fiumicino—Continued				Section B, Maccarese—Continued			
Sept. 9.....	161	18	76	.47	138	26	152	1.10
16.....	171	20	132	.77	163	18	102	.63
23.....	164	14	70	.43	149	23	111	.74
30.....	174	11	36	.21	150	12	35	.23
Oct. 7.....	158	7	11	.070	159	6	9	.057
14.....	167	3	4	.024	156	6	16	.10
21.....	167	2	6	.036	157	17	36	.23
28.....	154	0	0	0	157	16	49	.31
Nov. 4.....	149	1	2	.013	153	8	24	.16
11.....	162	0	0	0	131	6	27	.21
18.....	164	0	0	0	146	3	16	.11
25.....	133	0	0	0	135	1	5	.037

pigsties on the Isola Sacra within 300 meters of Lago di Traiano were also found positive and had to be resprayed.

The only known specific effort to control anopheles breeding in the test area during 1945 was made by the estate manager who raised the level of Lago di Traiano and cleared the margins. Nevertheless, larvae were found fairly frequently throughout the season in the canal joining the lake and the sea, and the probable source of the adults was eventually discovered. The ruined storehouses of the old Roman port near the lake had not been sprayed, because searches during the hibernation season of 1944-1945 led to the assumption that *A. labranchiae* did not rest there. A careful search of these ruins in the summer of 1945, however, revealed adult anopheles resting on the walls of the dimly lighted chambers. These mosquitoes might well have completed their life cycles without recourse to human blood meals, since livestock grazed at night in the vicinity. There were no human habitations within the direct line of flight between these storehouses and the canal. In the Acilia-Ostia section anopheles were found breeding late in the season in the Tiber River itself.

Maccarese was the last section to be sprayed; and the work there was not completed until June 15, after the breeding season was well advanced, which may account for the higher initial mosquito density. There were also 80 hectares (195 acres) of rice in this section, where larvae were found occasionally until the water was shut off in September. The premises harboring adult anophelines were located in the vicinity of one or another of these plantations, and their number decreased when the fields became dry.

Postspraying incidence of malaria.—The trend of annual malaria morbidity rates in the six sanitary units for which data were obtained from the Health Department of the Commune of Rome is shown for the years 1936-1945 in

Figure 5.² A sharp rise in incidence occurred in 1944 which was most conspicuous in the units of Ostia Antica and Fiumicino. In 1945 the rates dropped in four of the six units but they continued to rise in Maccarese and Palidoro.

The monthly distribution of malaria cases in all six units, including Ostia Lido, for the years 1944 and 1945 is shown in Table 5 and Figure 10. Late in 1943 many of the civilians in the Delta were evacuated by the Germans, con-

TABLE 4

Tiber Delta, 1945: Weekly captures of anopheles larvae per 20 dips in all sections

WEEK ENDING	STATIONS		ANOPHELES LARVAE	
	Examined	Positive	Captured	Per station
<i>1945</i>				
May 27.....	59	22	35	.59
June 3.....	77	14	59	.77
10.....	140	27	104	.74
17.....	200	50	346	1.73
24.....	203	38	190	.94
July 1.....	153	28	303	1.98
8.....	192	38	354	1.84
15.....	310	49	385	1.24
22.....	278	44	379	1.36
29.....	270	26	311	1.15
Aug. 5.....	270	26	250	.93
12.....	291	26	266	.91
19.....	254	22	182	.72
26.....	276	31	283	1.03
Sept. 2.....	275	33	307	1.12
9.....	273	25	278	1.02
16.....	276	30	315	1.14
23.....	262	32	255	.97
30.....	258	31	279	1.08
Oct. 7.....	263	30	266	1.01
14.....	258	23	146	.57
21.....	265	18	153	.58
28.....	268	19	174	.65
Nov. 4.....	228	9	55	.24
11.....	272	9	86	.32
18.....	261	4	33	.13
25.....	245	3	38	.16

sequently, in the early months of 1944 only a few scattered cases were reported. With the arrival of the Allies people returned to their homes, and an explosive outbreak of malaria occurred which reached a peak of 1,242 registered cases in August. The subsequent rapid decline may have been accelerated by measures taken to protect the Allied troops.

² Sanitary units, Acilia, Ostia Antica and Fiumicino, were within the treated area; about 60 per cent of the population in the Ponte Galera unit and 70 per cent of that in the Palidoro unit resided in the test area, while four or five premises belonging to the Maccarese unit were situated outside the treated section.

An unusual monthly distribution of malaria cases may be noted in 1945. There was an abrupt rise in incidence in January that continued to a peak of

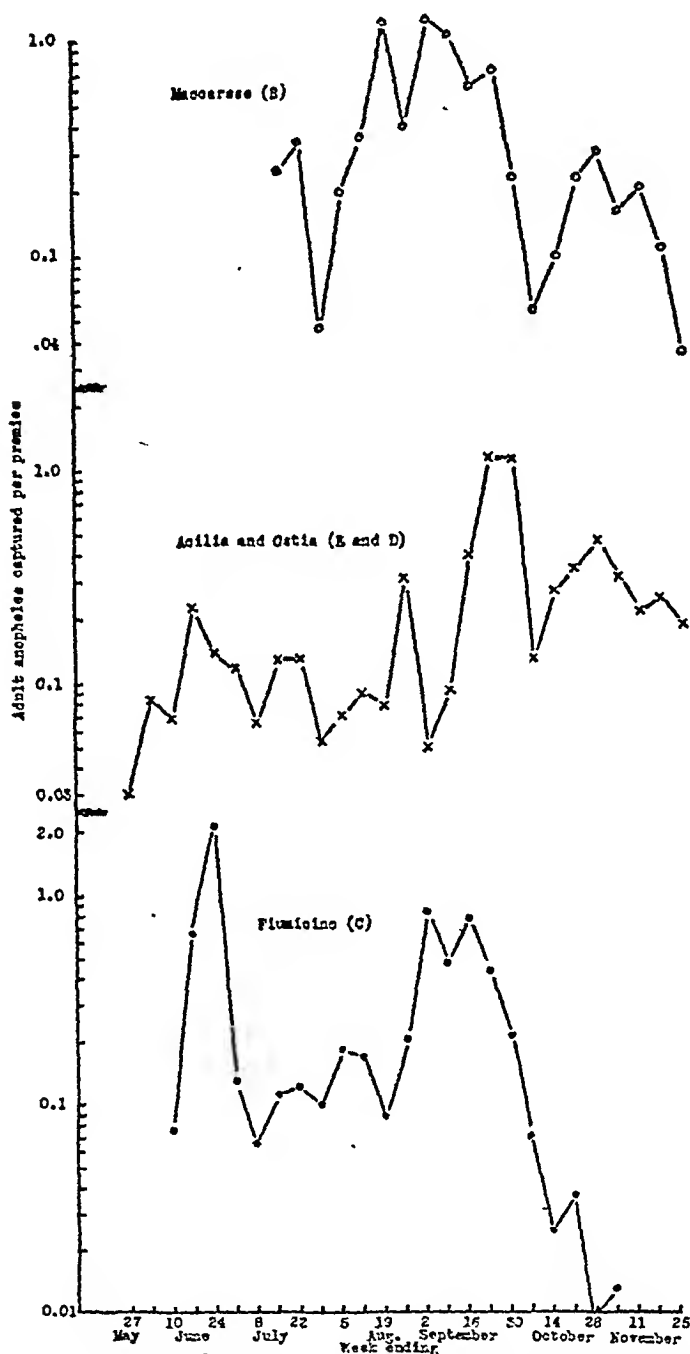


FIG. 8. TIBER DELTA, 1945: WEEKLY CAPTURES OF ADULT ANOPHELES BY SECTION

587 cases in March, which was two and one-half months before normal seasonal transmission could have affected the picture. Fewer cases were reported in

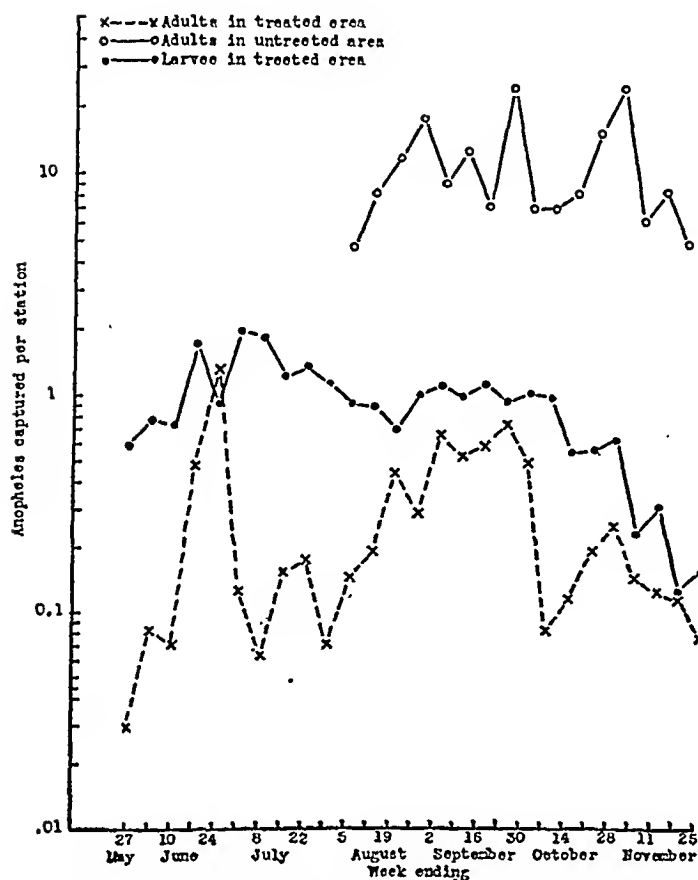


FIG. 9. TIBER DELTA, 1945: WEEKLY CAPTURES OF ANOPHELES (LARVAE AND ADULTS) IN TREATED AND UNTREATED AREAS

TABLE 5

*Tiber Delta: Malaria cases by month for the years 1944-1946**

MONTH	1944	1945	1946
January.....	3	162	34
February.....	2	283	37
March.....	7	587	27
April.....	5	538	
May.....	16	462	
June.....	67	400	
July.....	865	333	
August.....	1,242	224	
September.....	672	108	
October.....	322	52	
November.....	111	21	
December.....	62	5	

* Data from the Health Department of the Commune of Rome.

April, and the number continued to fall during succeeding months of the year. The customary summer peak in August failed to appear. The reasons for this

are believed to have been (1) the appearance of late onsets and winter and spring relapses among persons infected in 1944 and (2) the absence of adult anopheles in 1945 in sufficient numbers to cause the usual summer rise in the number of new infections.

Every effort was made by inspectors and competent staff members to discover instances of recent infection. One new malaria infection was verified in a three-months-old infant at Fregene, whose mother was reported to have had a relapse at the time of delivery. A thorough search of the neighborhood revealed a few anopheles in a small pigsty which had not been sprayed.

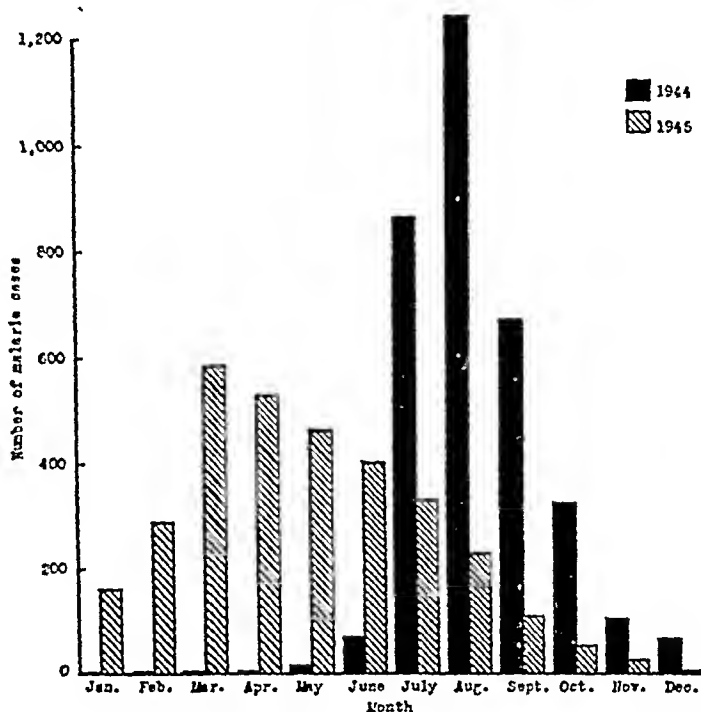


FIG. 10. CASES OF MALARIA REPORTED FOR THE TIBER DELTA IN 1944 AND 1945 BY MONTH

The following statement which summarizes the results of the application of DDT in the Tiber Delta in 1945 is by Professor A. Missiroli (3) who has studied malaria in this area for many years:

"Non un caso di malaria primitiva si é verificata nel Delta del Tevere, ed Ostia ha conseguito una salubrità che non aveva mai visto da duemila anni in poi, cioè da quando si ebbe l'invasione della Malaria in Italia."²

As another illustration of the effective use of house spraying with DDT Figure 11 is given. Professor Missiroli undertook to check the epidemic at Fondi, located between Castel Volturno and the Tiber Delta test areas, during

²"Not one case of primary malaria has been verified in the Delta of the Tiber, and Ostia has achieved a healthiness the like of which has not been seen there for some two thousand years, that is, since the invasion of Italy by malaria."

the second half of June, 1945. A sharp break in the number of new infections was noted 20 days later (3). Immediate and perfect antilarval work could not have achieved such quick results.

Residual effect of DDT.—When the Tiber Delta project was planned, it was hoped that a single application of insecticide would suffice to keep anopheles density below the level required for transmission of malaria for a full season. Field searches in the Acilia-Ostia sections in February 1946 failed to reveal mosquitoes in treated buildings. The intensive search at this time, however,

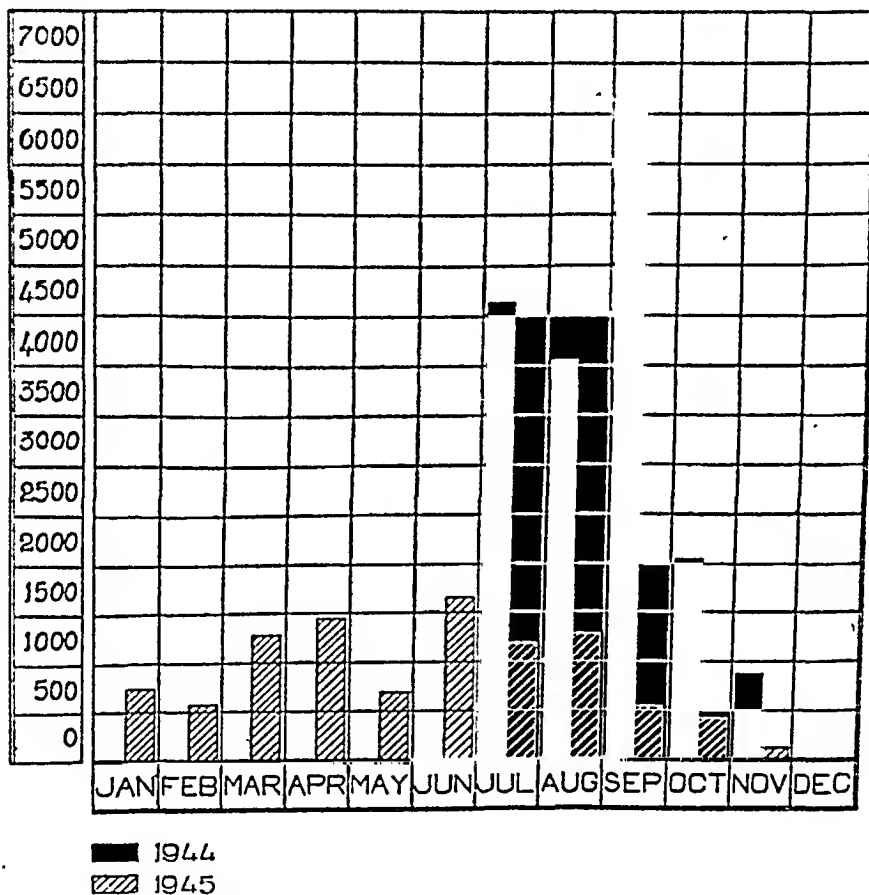


FIG. 11. CASES OF MALARIA REPORTED FOR FONDI
After A. Missiroli

led to the discovery of *A. labranchiae* in many unexpected places, such as empty silos never occupied by animals, open storage sheds, wood-piles near animal shelters, hollow logs, bundles of cut bamboo, etc. No *labranchiae* had been found resting under bridges or in culverts during the hot summer months of 1945, but they were discovered in such places in the late fall immediately preceding the period of hibernation.

The work of previous investigators.—At Castel Volturno and in the Delta of the Tiber it was observed that the failure to spray a pigsty or other building

might be reflected in the number of anopheles caught in premises at some distance, and that the spraying of such neglected shelters would result in the disappearance of anopheles from these other premises. Furthermore, Professor Missiroli found in 1945 that the spraying of farm buildings and those at the periphery of such towns as Fondi was sufficient to halt the rise of malaria within the towns.

In 1935 Park Ross (4) reported the results of three years of control of malaria in Zululand, Natal and South Africa through the weekly spraying of native huts with pyrethrum. Russell and Knipe (5) following some preliminary work by Covell *et al.* (6), undertook experiments in India to determine the efficacy of spray insecticides in the control of malaria in areas where *A. culicifacies* is the principal vector. Their results were impressive.

Other advantages of DDT application in the Tiber Delta.—In the light of the results obtained by other workers, it is believed that a greater reduction was achieved in anopheles density in the Delta than was needed to prevent transmission of malaria. When malaria control is the only consideration a more limited program may be planned at less cost. There were, however, definite advantages achieved by the Delta program in the destruction of all insects inside the premises. Freedom from house-flies, and sand-flies, bedbugs, cockroaches and fleas makes a big impression on people in treated areas and creates a public demand for respraying the following year and for the extension of spraying to neighboring areas. One may conjecture that eventually anopheles control may become a part of a routine program for the elimination of all household insect pests, requiring no special budget and no highly trained staff. The most urgent study now remaining is the determination of what malarious areas exist, if any, where residual DDT alone will not prevent transmission.

Acknowledgements.—In addition to the collaboration received from persons whose names have been mentioned, it is a pleasure to record that important contributions were made by the following to the studies described: Col. P.F. Russell, director, Malaria Section, Allied Control Commission in Italy, 1944; Lt. Col. Justin Andrews, malariologist, MTOUSA, 1944; Lt. Col. Richard M. Young and Capt. R. A. Elliot, 12th General Hospital, United States Army; Capt. L. M. Klashman and Sgt. C. R. Collins, 137th Malaria Control Detail; Capt. R. A. Fisher, Lt. A. W. Ziegler and Sgt. C. S. Black, 15th Medical Laboratory; Lt. John S. Wehrle and Sgt. Guy D'Aleo, 2675th Regiment, Allied Commission; Professoressa Lidia La Face and Sig. Francesco Neri, Istituto Superiore di Sanità; Rome; Drs. Francesco Kongo and Luigo Aloy of the Typhus Control Section of the Allied Control Commission; and Drs. H. W. Kumm and F. S. Markham of The Rockefeller Foundation Health Commission.

SUMMARY AND CONCLUSIONS

In 1944 and 1945 a 5 per cent DDT-kerosene spray was applied to the walls and ceilings of all buildings in specified areas of the Bonifica di Castel Volturno on the Italian coast just north of Naples. Results in 1944 were so promising that in 1945 a more extensive project was launched in an area of approximately

120 square miles in the Tiber Delta, where a 6.5 per cent solution of DDT in kerosene was applied once at a rate of approximately 200 milligrams per square foot to the interiors of all human habitations and animal shelters between February 27 and June 15. The objective was to determine the residual effect of the spray upon anopheline density in the absence of other control measures.

The organization of the field staff, the preparation of the insecticide and the equipment and methods employed in its application are described.

Routine searches for *A. labranchiae* adults and larvae in Castel Volturno and in the Tiber Delta indicated that density had been greatly reduced. A year after treatment no anopheles were found in previously sprayed buildings examined in the Delta.

In Castel Volturno a significant reduction in the parasite rate of school children occurred during the 16-month period of observation. While malaria morbidity in the Delta in 1945 was higher than in prewar years, its distribution by months showed a rapid rise to a peak in March and a continuous drop thereafter, indicating that most, if not all, of the cases reported came from infections of the previous year. The usual summer rise in incidence failed to appear.

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BOOK REVIEW

Practical Malariaology. PAUL F. RUSSELL, M.D., M.P.H., Colonel, M.C., A.U.S., Parasitology Division, Army Medical School, Field Staff, International Health Board, Rockefeller Foundation, LUTHER S. WEST, Ph.D., Head of Biology Department, Northern Michigan College of Education, Major, Sn.C. A.U.S., and REGINALD D. MANWELL, Sc.D., Professor of Zoology, Syracuse University, New York, Captain, Sn.C. A.U.S. Foreword by RAYMOND FOSDICK. Pp. i-xix, 1-684. W. B. Saunders and Co., Phil. and London. 1946. Illustrated.

In this beautifully printed and illustrated volume the authors have furnished public health workers and physicians a work which embodies their personal experience in malariology and the results obtained by others, in a practical manner. As stated in the Preface: "It is designed to give clinical, laboratory, and field information about malaria, capable of being put to use in daily practice. The design of the book is not encyclopedic or bibliographic, nor is it synoptic." This statement should be borne in mind in a consideration of the work from a critical standpoint, for while the subject of symptomatology, and the clinical aspects of malaria, are adequately considered, the practitioner of medicine will not find in this work an encyclopedic discussion of this phase of the subject. It is essentially a work devoted to the diagnosis and prevention of malarial infections and hence of very special value to the public health worker in malarious localities.

There is an excellent "Historical Introduction" followed by Section I of the book, devoted to a consideration of the plasmodia causing malaria in both man and the lower animals. This section is headed "The Parasite" but as malaria in both the human and animal host is caused by numerous species of plasmodia it would appear that this section would better be headed "The Parasites." In discussing the "exoerythrocytic cycle" of the plasmodia the authors do not accept the demonstration of this cycle in man although several excellent authorities have described such a cycle but they call attention to the fact that such a cycle would explain several still unsolved problems regarding the evolution of malarial infections in the human host. The colored plates illustrating this section are beautiful and accurate, especially the ones comparing the thick and thin stained films used in diagnosis. The descriptions of the morphology of the various plasmodia are adequate but it would appear to the reviewer that an unnecessary amount of space is occupied by descriptions of avian, monkey and mammalian malaria although, in some localities, the importance of such infections may warrant the space occupied by the illustrations and descriptions devoted to this phase of malariology.

The chapter devoted to "Laboratory and Field Technic" is excellent and complete directions are given regarding the technique of the complement fixation test which is assuming more and more importance in the diagnosis of malaria.

Section II of the book considers the mosquito under the following headings: Morphology, Taxonomy, and Life Cycle, Mosquito Bionomics, Distribution, Field Technic, Laboratory Technic. This section covers 118 pages and is the most complete, up-to-date, and valuable discussion of the subject that has come to the attention of the reviewer. It is especially notable because of the clear and detailed descriptions for the dissection of mosquitoes and the handling of living specimens, for experimental purposes.

Section III is devoted to a consideration of malaria from the standpoint of the human host of the parasite. The pathology of malaria is adequately discussed as is the symptomatology, treatment, and immunity, latency and relapse. To the clinician the discussion of the symptomatology and clinical aspects of the various types of malarial infection may be somewhat disappointing, as only about twelve pages are devoted to this portion of the subject, but it should be remembered that this book is not a work upon clinical malariology but is essentially one devoted to the public health aspects of malaria. The discussion of treatment is excellent and the authors state "that not much difference will be seen between short courses of quinine and the usual atabrine treatment in regard to the occurrence of

vivax relapses, although with atabrine a longer average interval occurs between the end of treatment and a relapse.

In Section IV the malarial infections are considered from the standpoint of the community. This section includes the epidemiology of malaria, climatological factors, malaria epidemics and malaria surveys. The most valuable of these chapters is that upon malarial surveys, covering twenty-six pages. It is the most up-to-date and comprehensive discussion of the subject that has come to the attention of the reviewer and should prove of inestimable value to all interested in the prevention of malarial infections.

Section V is devoted to a thorough consideration of the control of malarial infections and, as would be expected, is the largest section of the book, containing 182 pages, as the work is essentially one devoted to the public health aspects of malaria. This section discusses the classification of control methods, control of the parasites, larvicides, drainage and filling, control of adult mosquitoes, control of man, and malaria control under military conditions. All of these phases of malaria control are adequately covered and this portion of the book will be of the greatest value to workers in this field of disease prevention. The use of DDT in killing malaria mosquitoes is thoroughly considered and quinine and atabrine prophylaxis reviewed and discussed, the authors rightly giving preference to atabrine, stating "It is the best drug now available for clinical prophylaxis and has been invaluable for military personnel." Regarding the use of atabrine for suppression of malaria in military operations the authors say "The tremendous advantages of carefully controlled atabrine chemoprophylaxis in a military unit are obvious: no deaths from *falciparum* malaria, no black-water fever, few clinical attacks, and few gametocyte carriers in any group which faithfully carries out an atabrine suppressive regimen."

The reviewer regrets that lack of space forbids a more thorough review of this book but it is one that should certainly be in the hands of every public health worker and physician having to do with malarial infections. The authors are to be congratulated upon this most excellent consideration of the subject. The book is printed on fine paper and the illustrations are numerous and valuable.

CHARLES F. CRAIG

THE PERIODICITY OF MICROFILARIAE IN TWO PATIENTS WITH FILARIASIS ACQUIRED IN THE SOUTH PACIFIC¹

DON E. EYLES,² GEORGE W. HUNTER, III³, AND VIRGINIA G. WARREN⁴

INTRODUCTION

During World War II many of our troops were stationed in areas where filariasis is endemic and there has been considerable uncertainty regarding proper designation of the type of filariasis resulting from this exposure. Accurate classification is made difficult because there are several varieties of the disease and in addition, these frequently exist in the same area. For instance, filariasis due to *Wuchereria bancrofti* (Cobbold) has a so-called periodic and non-periodic type, and in some regions one or both of these types are encountered together with filariasis due to *Wuchereria malayi* (Brug).

While numerous attempts have been made to study the various aspects of this disease in our troops, little work has been done on periodicity because of the scarcity of patients exhibiting microfilariae in their blood. Recently, however, it has been possible to study two men who were exposed while they were in the Society and the Tonga Islands. This paper considers the microfilarial periodicity displayed by these two patients with filariasis due to *W. bancrofti*.

CASE HISTORIES

The case history of patient A, a white male, age 25, 603rd Field Artillery Battalion, has been given in detail in a report by Eyles and Most (1947). A brief recapitulation is given here. This man enlisted in 1940 and spent three months on maneuvers in Puerto Rico; about one-half of this time was passed on shipboard outside the harbor of San Juan. Malaria discipline both on the boat and ashore was very strict so it is believed that little, if any, opportunity existed for exposure to mosquitoes carrying filariasis. Subsequently, one and one-half years were spent on Bora Bora in the Society Islands followed by seven months on Guadalcanal and Bougainville in the Solomons. On Bora Bora the patient gave a history of prolonged close contact with the natives, many of whom had filariasis. Mosquitoes were reported as a constant annoyance.

Patient A returned to the United States in May of 1944. Approximately one year later microfilariae were demonstrated in the peripheral blood (about 19 months after the first presumable exposure period).

Patient B, a white male, age 23, was inducted into the Army in 1940. Prior to this time he had not been outside of the continental limits of the United States. He left the United States in April, 1942, and served for one year in a battalion

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of the 134th Field Artillery on Tonga Tabu in the Tonga Islands. After a month in Townesville, Australia, he spent approximately nine months on Woodlark Island (just east of New Guinea), was then transferred to Sydney, Australia, and subsequently to the United States in July, 1944.

While on Tonga Tabu, the patient lived in an area heavily infested with mosquitoes and mingled with the natives during the day and night. During the first week on Tonga Tabu mosquito bars were not available and the patient reports that even when they later became available they were penetrated by mosquitoes, mites, and "sandflies". Repellents were not used extensively.

In December, 1943, he was suspected of having filariasis. Symptoms included swelling and tenderness of the left axillary nodes, testicles and inguinal glands. The patient suffered recurrences of this at intervals until his discharge from the Army in October, 1945. Attacks were aggravated by physical exercise. He had a positive skin test with *Dirofilaria immitis* and *W. bancrofti* antigens in November, 1944.

Microfilariae were recovered by the Knott technique (1939) on August 9, 1944. They persisted throughout his hospitalization and were still present in October, 1945, when the patient returned to civilian life.

METHODS

Microfilarial counts were made by direct examination of stained or fresh smears made from finger punctures except in the first three series of counts on patient B when Knott's concentration technique was used because of the small number of microfilariae in the peripheral blood. For these counts 1 cc. samples were used.

After the number of microfilariae in the blood of patient B had increased, the contents of five Sahli pipettes, totalling 100 cmm., were counted after proper fixation and staining. Counts on patient A were made by examining 25 cmm. of fresh blood. A 5 cmm. pipette was used and five such samples were examined in each instance. On the graphs and charts all counts on both patients are converted to number of microfilariae per 100 cmm.

FINDINGS AND DISCUSSION

The microfilariae, found in greatest numbers during the day in the peripheral blood of these two patients, were carefully studied microscopically. They appeared to be morphologically indistinguishable from microfilariae of the nocturnal type of *W. bancrofti*.

Figure 1 presents graphically the results of microfilarial counts on both patients. The upper graph is the mean of four series of counts on patient A during July, 1945. The lower one is the mean of eight series of twenty-six hour counts taken at two hour intervals on patient B while he was on day duty. In both instances the mean daily counts are indicated by dotted lines. It will be noted that a much larger number of larvae were present in the peripheral blood of patient A, his daily mean count being 380 as compared with 9.5 per 100 cmm. for patient B. Despite this quantitative difference the graphs show marked simi-

larity. Peak counts for both patients are diurnal, and the ratios of the mean high and low counts are comparable, being 3.4 in the case of patient A and 2.3 for B.

Figure 2 gives the results of three series of counts each extending over a period of 48 hours or more on patient A. The first series taken July 16-18, 1945, was obtained by taking samples hourly during the afternoon hours of high microfilarial density, every two hours during the period of intermediate density and

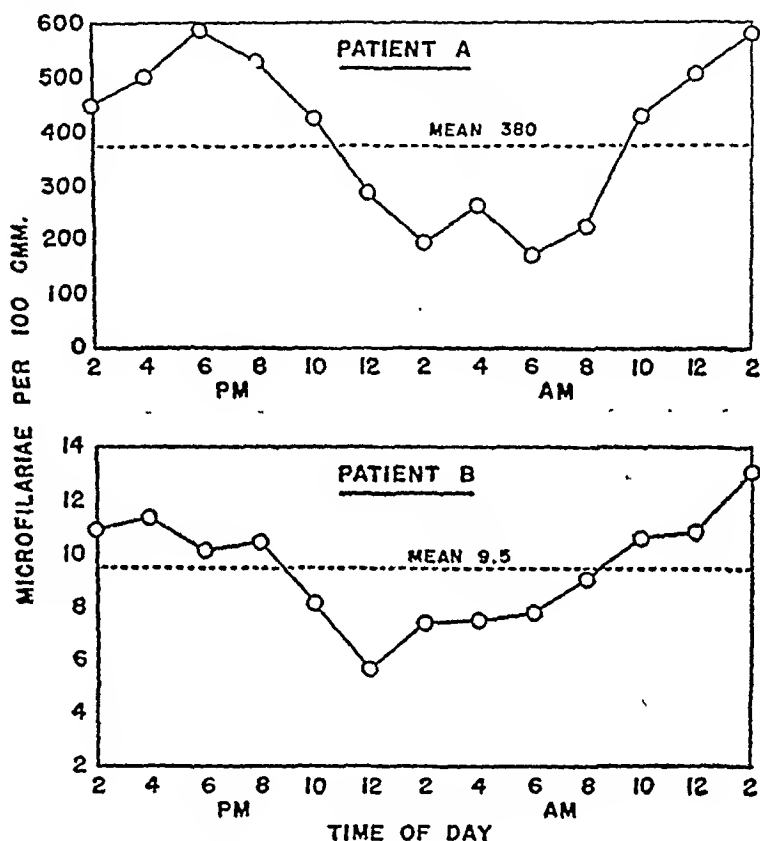


FIG. 1. COMPARISON OF THE MEAN OF THE MICROFILARIAL COUNTS ON PATIENTS A AND B

The upper graph is based upon a series of four 26 hour counts on patient A during July, 1945. The lower graph presents a series of eight 26 hour counts on patient B taken over a 14 month period. Dotted lines are mean daily counts.

every four hours during the night when the count was lowest. This method of sampling involved the least disturbance of the normal rest of the patient. The data from this series of samples are in essential agreement with those obtained from a second series of consecutive hourly samples over a 60-hour period taken July 24-26, 1945.

The similarity in the results of these two series suggests that the more frequent interruption of sleep which resulted during the second series had little effect upon the periodicity of the microfilariae. During the third series, taken February 13-15, 1946 (precisely four months after treatment with stibanose), counts

taken every two hours over a period of 48 hours showed some increase over the two previous counts.

Examination of the graphs in figure 2 reveals that about 10:00 a.m. the number of microfilariae exceeds the mean daily count. It falls below the mean between 10:00 p.m. and midnight. The absolute high occurred between 12 noon and 8:00 p.m. and the absolute low between 2:00 a.m. and 8:00 a.m. It

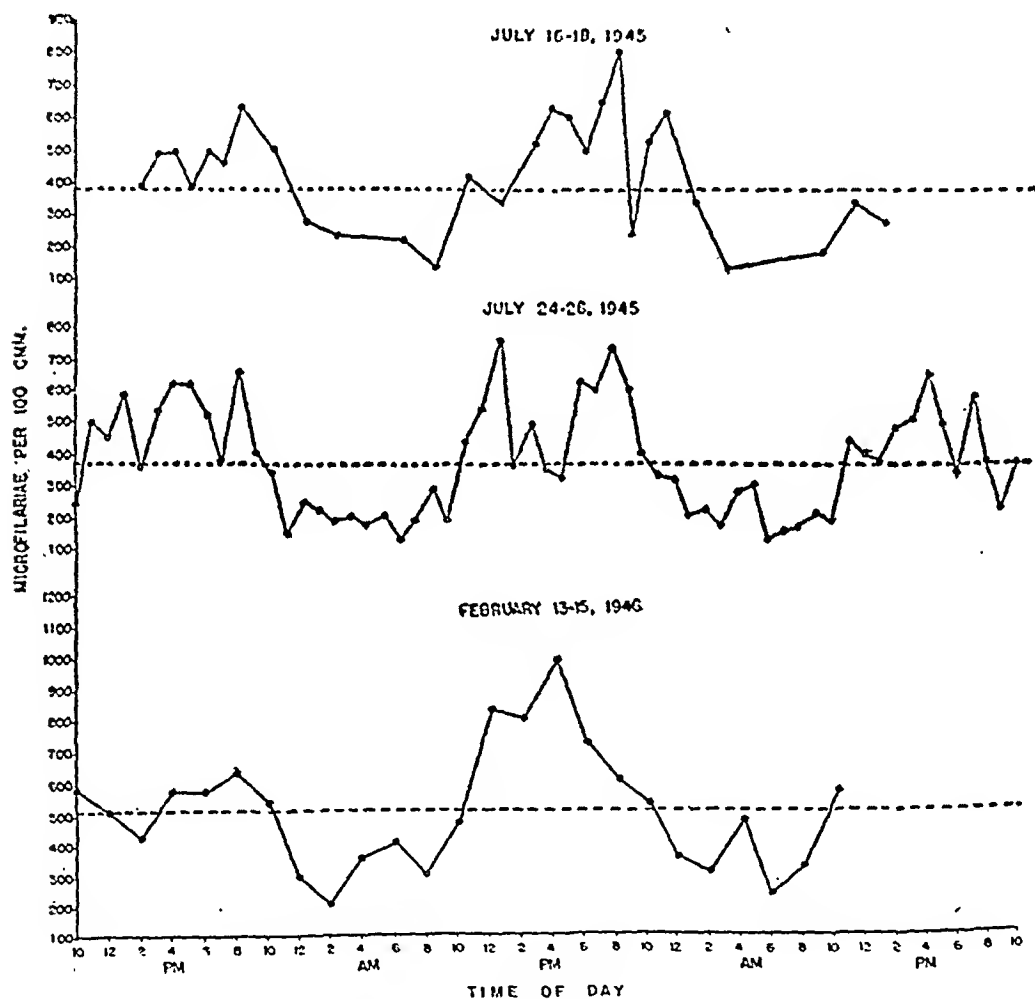


FIG. 2. MICROFILARIAL COUNTS ON PATIENT A

Dotted lines represent mean daily count.

is worthy of note that numerous counts on patient A over a seven month period (July, 1945 to February, 1946) between the hours of 2:00 p.m. and 4:00 p.m. showed only slight variation. It will be noted, however, that the mean daily count had increased somewhat by February, 1946.

The data presented in figure 3 represents the mean of counts taken on patient B on ten different occasions. Counts were taken every two hours over 26 hour periods. The first series was begun on August 28, 1944 (19 days after the first microfilariae were found) and the last count was made on October 16, 1945. Eight of these counts were completed while the patient was active during the

day and sleeping during the night, and two were taken while the patient was on night ward duty. Results of both series are tabulated in table 1.

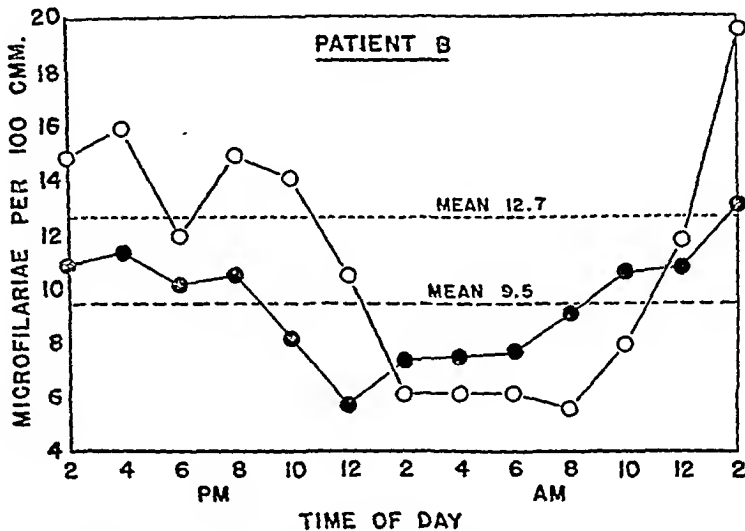


FIG. 3. MEAN COUNTS OF MICROFILARIAE IN BLOOD OF PATIENT B

The continuous line with black circles represents counts when patient was active during the day; the broken line represents counts made when patient was on night ward duty. Top horizontal broken line represents mean count when on night duty; bottom, when on day duty.

TABLE 1
Ten series of microfilarial counts on patient B
Number of Microfilariae per 100 cmm.

SERIES NUMBER	DATE	TYPE OF DUTY	HOUR														MEAN OF SERIES
			2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 M	2 a.m.	4 a.m.	6 a.m.	8 a.m.	10 a.m.	12 N	2 p.m.		
I	*Aug. 28-29, 1944	Day	2.0	1.0	1.5	2.0	2.9	1.7	1.3	1.5	1.5	1.0	1.9	1.7	1.8	1.6	
II	*Sept. 28-29, 1944	Day	5.9	7.4	3.1	3.8	3.7	3.3	2.4	3.7	5.4	2.9	5.0	1.8	7.8	4.3	
III	*Oct. 26-27, 1944	Day	5.0	2.8	2.6	2.2	4.6	2.4	2.0	2.8	3.2	3.4	1.6	4.7	4.6	3.2	
IV	Dec. 18-19, 1944	Day	15.0	11.0	10.5†	10.0	8.0	4.0	5.0	4.5†	4.0	16.0	18.0	11.0	17.0	10.3	
V	Feb. 1-2, 1945	Night	16.0	15.0	11.0	11.0	11.0	8.0	6.0	6.0	7.0	6.0	12.0	12.0	19.0	10.8	
VI	Mar. 13-14, 1945	Day	15.0	6.0	10.0	19.0	7.0	5.0	5.7†	6.3†	7.0	7.0	5.0	14.0	22.0	9.9	
VII	Apr. 16-17, 1945	Night	14.0	17.0	14.0	19.0	18.0	14.0	7.0	7.0	6.0	5.0	4.0	12.0	20.0	12.1	
VIII	May 9-10, 1945	Day	17.0	19.0	20.0	14.0	10.0	5.0	16.0	5.0	14.0	7.0	11.0	17.0	22.0	14.4	
IX	July 5-6, 1945	Day	16.0	31.0	21.0	16.7†	12.3†	8.0	11.0†	14.0	16.0	25.0	31.0	25.0	18.0	18.8	
X	Oct. 15-16, 1945	Day	12.0	12.0	13.0	16.0	17.0	16.0	16.0	13.0	12.0	11.0	12.0	12.0	12.0	13.4	
																MEAN OF ALL COUNTS	
Hourly Mean			Day	11.0	11.3	10.2	10.5	8.2	5.7	7.4	7.6	7.9	9.2	10.7	10.9		13.2
			Night	15.0	16.0	12.5	15.0	14.5	11.0	6.5	6.5	6.5	5.5	8.0	12.0	19.5	12.7

* Knott technique employing 1 cc. of venous blood used for counting microfilariae.

† Samples unsatisfactory for counting. These are interpolated values.

The ward work was relatively light and the patient was able to secure considerable rest and some sleep during the early morning hours. Consequently it is not surprising that these two graphs should appear to be essentially similar.

As indicated in figure 3, counts made when the patient was on night duty were slightly higher than those made when the patient was diurnally active. This apparent difference is due to the fact that the night counts were made in February and April, 1945, when the microfilariae were present in considerable numbers, while the other series included the first three counts which were made in August, September and October, 1944, when the microfilarial density was very low.

When the patient was active during the daytime his microfilarial counts fell below the mean shortly after 8:00 p.m. and remained below for about twelve hours. When on night duty, counts fell below the mean shortly after 10:00 p.m. and passed above it about twelve hours later.

Examination of table 1 reveals that during the observation period of about 14 months, the number of larvae increased from 1 to a peak of 31 per 100 cmm. of blood on July 5 and 6, 1945, with an average of 18.8 for the 26 hour observation period. Three months later, the peak count had dropped to 17 per 100 cmm. and the daily mean to 13.4. At no time had the patient received specific drug therapy. Whether or not this represents a permanent decrease in numbers cannot be stated at this time.

The two strains of *Wuchereria bancrofti* from the Society and Tonga Islands discussed here are believed to represent infections of what has been termed the non-periodic type of filariasis bancrofti. Between 140° and 180° east longitude both this and the nocturnal form of the disease occur, while west of 140° east longitude all filariasis is nocturnal. East of 180° of longitude the so-called non-periodic type exists. (Both Bora Bora and Tonga Tabu are east of this meridian.) In view of the definite microfilarial periodicity observed in patients A and B, the term non-periodic or aperiodic, as applied to the microfilariae of *Wuchereria bancrofti* from the South Pacific Islands east of 180° longitude, seems to be an improper term. Likewise, the term "Pacific strain" is considered to be undesirable since some Pacific Island microfilariae are definitely of the nocturnal type. Furthermore, this term might be confused with Manson-Bahr's designation of *Wuchereria pacifica*, a name at present considered by most authorities to be a synonym for *W. bancrofti*. The authors suggest that, since the designation "non-periodic" is not properly descriptive, it should be discarded. Actually the term "diurnal" appears to be more descriptive and it is suggested as a more appropriate term.

This suggestion is based upon only two cases, both studied in the United States. Several individuals who have studied *W. bancrofti* clinically in the Pacific Islands have failed to note such periodicity. However, the constant presence of large numbers of microfilariae coupled with a low ratio between maximum and minimum counts would make the detection of such a cycle difficult unless counts were made on large samples of blood over a prolonged period of time and at relatively short intervals.

Bahr (1912), working in the Fiji Islands where both the periodic and diurnal types are found, presents a number of charts of counts made at four hour intervals. Many of these suggest a diurnal type of periodicity resembling those described for patients A and B.

SUMMARY AND CONCLUSIONS

The following points are based upon microfilarial counts of the blood of two patients parasitized with *Wuchereria bancrofti*.

1. Microfilariae were present at all hours of the day and night regardless of whether or not the parasitized individuals were awake or asleep.

2. In these two cases peak counts over 26 hour periods were diurnal, the peaks occurring between noon and 8:00 p.m.

3. Conversely, the lowest densities were nocturnal, occurring between midnight and 8:00 a.m.

4. The ratio of the mean high and low daily counts in both patients was low, being 3.4 in the case of patient A and 2.3 in the case of patient B.

5. In patient A the count over a seven and one-half month period showed some increase in numbers of microfilariae in spite of drug therapy with stibanose.

6. In patient B the peak density occurred 11 months after microfilariae were first demonstrated in the patient's blood. Three months and a half later the count showed a decrease, although the patient received no therapy.

7. It is suggested that the term "diurnal periodicity" might be found to be more appropriate than the terms "non-periodic" or "aperiodic" which appear widely in the literature.

1. The authors express their appreciation to the staff of the Moore General Hospital and especially to Major Harry Most for cooperation in this study.

2. This program is part of a series of papers on filariasis from both the U. S. Public Health Service and the Army Medical School.

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INFECTIVITY OF PACIFIC ISLAND WUCHERERIA BANCROFTI TO MOSQUITOES OF THE UNITED STATES*

DON E. EYLES,¹ AND HARRY MOST²

INTRODUCTION

The studies herein reported were undertaken to determine the susceptibility of mosquitoes of the United States to a South Pacific island strain of *Wuchereria bancrofti*. The strain of *W. bancrofti* discussed here is believed, from the history of the patient and the diurnal prevalence of microfilariae, to be representative of what has been termed the "non-periodic" type found in several South Pacific island groups (east of 180° longitude all filariasis is of this type; between 140° and 180° east longitude both non-periodic and nocturnal occur; and with certain exceptions west of 140° east longitude all filariasis is nocturnal. Bora Bora is situated near 150° west longitude.)

Comparison of the work of Bahr (1912) with that of other investigators (Newton, *et al.*, 1945) would indicate that this island strain may differ in its host relationships with mosquitoes; Bahr found *C. quinquefasciatus* (as *C. fatigans*) to be relatively insusceptible to a Fiji strain, whereas investigators of the nocturnal strain almost unanimously have found that *C. quinquefasciatus* is highly susceptible. The data presented here may also have a double significance, as: (1) Useful data indicating the potential vectors in this country are made available; and (2) The data may be applied with proper cautions in control work in the Pacific islands where *C. quinquefasciatus* is common along with the recognized vector, *Aedes variegatus*.

During the recent war, among thousands of troops stationed in various Pacific islands where filariasis is endemic or hyperendemic, many persons developed various signs and symptoms which necessitated their return to the continental United States for study of suspected filariasis infection. In most instances regarded as probable infection, the diagnosis was based on history, physical findings and skin test with *Dirofilaria immitis* or other antigen. In some, the diagnosis was proven by the presence of adult worms in biopsy material, and rarely by the presence of microfilariae in the blood. The clinical syndrome seen in the large number of men evacuated from these areas has been accepted as representing the result of infection with *Wuchereria bancrofti*. Experience during the past few years has led to the general conclusion that while the infection rate may have been high in this group of individuals, for the most part the infections were light and will rarely result in permanent clinical disease. It has been shown that recurrences of symptoms and signs become less frequent with the passage of time, and in a majority the infection has become completely in-

* Contribution from the National Institute of Health and the Moore General Hospital U. S. Army.

¹ U. S. Public Health Service, National Institute of Health, Division of Infectious Diseases.

² Major, Medical Corps, Army of the United States.

active within 2 years of leaving the endemic area. (Coggeshall, 1945, and experience at the Moore General Hospital.)

The incidence of positive blood smears in military personnel with filariasis is very low (less than 1 per thousand clinical cases). Consequently there would seem to be only small likelihood of transmission of this infection to American mosquitoes with subsequent establishment or spread of filariasis in this country. However, the opportunity of gathering information as to susceptibility of United States mosquitoes to *Wuchereria bancrofti* of Pacific origin was at hand and was explored.

CASE HISTORY

The patient concerned is a 25 year old white male, born and reared in Pennsylvania. During basic training a period of 3 months was spent on maneuvers in Puerto Rico, six weeks of this time being spent on board ship outside the harbor in San Juan and 6 weeks in an unpopulated training area. Insect repellents and mosquito bars were used in Puerto Rico with regularity, and no time was spent overnight in San Juan or visits made at night to any villages. Eighteen months were subsequently spent by the patient on Bora Bora in the Society Islands, and 7 months on Guadalcanal and Bougainville in the Solomons. The patient returned to the United States on May 12, 1944. History prior to military service was not significant.

While on Bora Bora there was prolonged close contact with natives, many of whom had filariasis. The biting of mosquitoes was noted continually. The patient had an appendectomy on Bora Bora in March, 1942, suffered from chronic otitis media for about 6 months, and on one occasion had diarrhea and vomiting for 2 days. While in the Solomon Islands the patient had 4 verified attacks of vivax malaria.

While stationed at Green Haven, N. Y., commencing in February, 1945, the patient experienced chills and fever which lasted for 1 or 2 days and recurred about twice monthly for several months. On numerous occasions during these episodes of chills and fever the patient reported to a dispensary for blood smear examinations. On no occasion were malaria or other parasites found, nor was any specific treatment given. On May 14, 1945 following an episode of chills and fever, the patient reported to the station hospital at Green Haven, New York, where blood smears were made. At this time, microfilariae were found in the blood and the patient was hospitalized the following day. He was subsequently transferred to Moore General Hospital where he arrived on May 30, 1945. The history obtained was that already presented.

Physical examination was not remarkable except for discrete flat, soft lymph nodes enlarged to a maximum of 1 cm. in diameter found in the right axilla, right femoral and both inguinal regions.

The urine, stool, serology, and complete blood counts were normal. Microfilariae were present in the peripheral blood. Skin test with *Dirofilaria immitis* antigen was positive.

The patient was completely asymptomatic, and volunteered to undergo the

various mosquito infectivity experiments to be presented. These mosquito feedings were made between June 2, 1945 and February 14, 1946.

PARASITOLOGY

The morphology of the microfilariae of *Wuchereria bancrofti* from Bora Bora is as far as the authors can determine identical to that of the nocturnal type. The only difference between the two lies in the periodicity. Studies of periodicity on this patient and a patient from the Tonga Islands is the subject of another paper (Eyles, Hunter, and Warren).

A comment is warranted here concerning the relationship of periodicity to vector. In the case studied, the number of microfilariae is probably sufficient to infect mosquitoes at all hours as counts on this patient varied from 2 to 8 microfilariae per cmm. In addition (Eyles, Hunter, and Warren) the period of highest microfilarial density extended from true daylight in the afternoon until after sunset, thus allowing exposure to both day and night biting mosquitoes.

During the study of the patient, counts of microfilariae were made in connection with each mosquito feeding. That little change in the numbers took place is demonstrated by the fact that the mean of counts made during June and July was 4.8 per cmm. as against a mean of 5.35 per cmm. in September (overall mean 5.05 per cmm.).

In the mosquito host the parasitology was similar to that described by other investigators. Immediate penetration of the thoracic muscles was followed by development to the infective larva, the first stage in the thorax being characterized by a sausage-like shape with a terminal process. Advanced stage larvae as used in this paper include those in the infective stage, early third stage, and late second stage, as in all species in which development progressed past the first stage infective or larvae nearing the infective stage were found. When complete development did not occur, the filariae almost invariably aborted in the first stage.

In regard to the ingestion of microfilariae, the authors observed on several occasions in the thoracic muscles of *Culex pipiens*, *Anopheles walkeri*, and *Anopheles punctipennis*, a greater number of the larvae than would be expected from the volume of blood taken. No explanations for this are advanced, but the discrepancy in numbers was so great in some cases that it could hardly be due to the absorption or extrusion of the plasma.

DETERMINATION OF SUSCEPTIBILITY

Technique. The technique of handling and feeding the mosquitoes followed closely that of Burgess and Young (1944), deviations being occasionally necessitated by the habits of the species being studied. Ordinarily, one mosquito was placed in each feeding container. In this regard, good feedings with *Culex pipiens* and *C. quinquefasciatus* were obtained by a modification of the above method, 20 to 40 adult female mosquitoes were enclosed in a cardboard cage about one inch by four inches in diameter. One face of this cylinder was covered by netting, the other by cardboard and the netting face was applied closely to

the skin of the patient. Mosquitoes not feeding on the first or subsequent trials were destroyed.

Mosquitoes were applied during the period of greatest microfilarial density, generally between 2:00 and 4:30 p.m., E.W.T. Counts of from 10 to 40 cmm. of blood were made at the time of feeding (with a few exceptions). Mean density at the time of feeding was 5.05 per cmm. (Standard deviation 1.3). Since density was thus uniform, no counts accompany the tabulations of individual lots. On a few occasions lots were applied in the early evening (7:00 to 8:00 p.m.).

TABLE 1

Species of mosquitoes fed upon patient showing microfilariae in peripheral blood stream

SPECIES	NO. LOTS	NO. APPLIED*	NO. FED	PER CENT FED	SOURCE OF MOSQUITOES†
<i>Culex pipiens</i>	8	580	100	17.2	1, Asheville, N. C.
<i>Culex quinquefasciatus</i> (Coastal)	6	384	117	30.5	1, 3 Coastal, S. C.
<i>Culex</i> sp. (Columbia, S. C.)	1	43	41	95.3	3, Columbia, S. C.
<i>Culex erraticus</i>	4	88	43	48.9	1, Orangeburg, S. C.
<i>Culex salinarius</i>	6	200	113	56.5	1, 3 Coastal, S. C.
<i>Anopheles walkeri</i>	6	146	73	50.0	2, 3 Coastal, S. C.
<i>Anopheles punctipennis</i>	5	402	265	65.9	1, Asheville, N. C. Spartanburg, S. C.
<i>Anopheles quadrimaculatus</i>	9	620	479	77.3	4
<i>Anopheles freeborni</i>	2	200	185	92.5	4, California
<i>Aedes triseriatus</i>	8	229	152	66.4	1, Asheville, N. C. Coastal, S. C.
<i>Aedes aegypti</i>	4	220	145	66.0	4
<i>Aedes atropalpus</i>	4	10	7	70.0	1, Austin, Texas
<i>Aedes atlanticus</i> or <i>tormentor</i>	5	94	26	27.7	2, Coastal, S. C.
<i>Psorophora ferox</i>	5	300	110	36.7	2, Coastal, S. C.
<i>Mansonia perturbans</i>	4	190	146	76.8	2, Coastal, S. C.

* In many instances mosquitoes which failed to feed were reapplied with a second lot which causes the percentage fed to be low in some cases.

† Index numbers refer to method of obtaining adults: 1—reared from wild caught larvae or pupae; 2—wild caught; 3—reared from eggs deposited by wild caught females; 4—insectary strains.

Those mosquitoes which fed were maintained between 76°–80°F., with a high relative humidity. Dilute sugar water (made from commercial syrup) was applied nightly until dissection.

Dissections were made mostly between the 10th and the 20th days, since about 15 days seemed the optimum time for complete development in susceptible species. If advanced development occurred, it could be noticed by the 10th day, and only dissections after 10 days are included in computing the proportions infected.

Dissections, after removal of the wings, abdomen, and legs, consisted of

placing the thorax and the head (including the proboscis) in separate drops of normal saline, the heads being removed by traction. After being thus placed the thorax was opened and the muscles teased out and apart with fine needles while being inspected under the dissection microscope (usual magnifications $\times 27$ and $\times 54$). Any worms seen were noted and the preparation then carefully examined under low power of the compound microscope ($\times 60$). Following the thorax examination, the whole head and proboscis were examined to see if infective larvae had emerged, this in turn being followed by teasing dissection of the head and proboscis parts.

Data on the number of lots, numbers applied and percentages fed, as well as details as to the origin of the mosquitoes, are summarized in table 1.

A large number of *Culex restuans* Theob. was applied to the patient but none would feed, although variations of the method of pre-treatment were tried. A lot of about 50 *Aedes sticticus* (Meig.) died within 72 hours after feeding on the patient. Since simultaneously fed and incubated lots of the other species used as controls survived normally, it was suspected this mortality might have been due to deleterious effects of the filaria larvae.

Discussion of results. Table 2 summarizes the results obtained by dissection. In the interpretation of the data included in this table consideration must be given not only to the percentage infectibility of each species but also the habits, prevalence and economic importance of the species, and the quantitative factor of degree of infection in susceptible mosquitoes.

Of the species tested, *Anopheles quadrimaculatus* (Say), *Anopheles freeborni* (Aitken), and *Psorophora ferox* (Humb.), at no time showed advanced larvae. In the two *Anopheles*, larvae had advanced to the thoracic muscles in only a few instances and in these instances development was aborted at an early stage. No larvae were noted in the thorax of *Psorophora ferox* at any time.

In one lot of *Aedes* it could not be accurately determined from the adult females if the species was *A. atlanticus* D. and K. or *A. tormentor* D. and K.; however, no specimens of this group were found infected with filaria larvae. In the region from which these were collected, *Aedes atlanticus* predominates.

Concerning the above four species which showed no infection, some specimens were tested simultaneously with specimens of other species which did show infections, thus showing that potentially infective larvae were present at time of feeding.

Of the species tested, *Culex pipiens* L. was most consistently susceptible both as to percentage and degree of infection. This species could, in consequence of its 83.5 per cent infection rate, become an efficient vector, since it breeds in a variety of situations, even in urban and suburban areas, and readily attacks man under some circumstances. *C. pipiens* is most often a nocturnal biter, but the hourly counts showed that the number of microfilariae in the peripheral blood remained high well into the evening. Furthermore, some microfilariae were found in the blood at all times.

Culex quinquefasciatus Say is perhaps the most consistently abundant pest mosquito in the South. It breeds close to habitations of man, often being found

in polluted waters and in artificial containers as well as a variety of other breeding places. This species was less susceptible both qualitatively and quantitatively than *Culex pipiens*, only 34.9 per cent being found infected with a mean number of 2.7 advanced larvae against 83.5 per cent with a mean of 9.3 larvae for the latter species (table 2). Nevertheless, owing to its close association with man, *C. quinquefasciatus* might still be of potential menace in many areas.

TABLE 2
Development of Wuchereria bancrofti in various species of mosquitoes

SPECIES	EARLY DEVELOPMENT OF LARVAE: 2-9 Days		LATE DEVELOPMENT OF LARVAE: 10 DAYS OR MORE AFTER FEEDING				TOTAL % INFECTIBILITY BASED ON LATE DISSECTIONS*	NO. NORMALLY DEVELOPING LARVAE PER SPECIMEN		Importance†
	Number mosquitoes dissected	Number showing normal development	Number mosquitoes dissected	No. showing advanced but not infective stage larvae	No. showing infective larvae only in thorax	No. with larvae in head or proboscis		Mean	Extremes	
<i>Culex pipiens</i>	2	2	79	6	5	55	83.5	9.2	1-31	1
<i>C. quinquefasciatus</i> (Coastal).....	0	0	106	8	11	18	34.9	2.7	1-9	1
<i>C. sp.</i> (Columbia, S. C.)..	0	0	37	2	2	27	83.8	7.5	1-23	
<i>Culex erraticus</i>	2	0	35	5	2	1	22.9	2	1-5	3
<i>Culex salinarius</i>	13	0	102	3	0	0	2.9	4	2-7	2
<i>Anopheles walkeri</i>	36	12	14	3	2	0	35.7	2.3	1-5	4
<i>Anopheles punctipennis</i> ..	11	0	118	0	2	1	2.5	5	1-9	3
<i>A. quadrimaculatus</i>	14	0	120	0	0	0	0.0			1
<i>Anopheles freeborni</i>	6	0	70	0	0	0	0.0			1
<i>Aedes triseriatus</i>	8	1	120	0	2	6	6.7	3.4	1-10	2
<i>Aedes aegypti</i>	0	0	90	0	1	2	3.3	3.3	1-6	1
<i>Aedes atropalpus</i>	1	0	6	0	1	0	1 out of 6		19	4
<i>Aedes atlanticus</i> or <i>tormenlor</i>	0	0	15	0	0	0	0.0			2
<i>Psorophora ferox</i>	0	0	71	0	0	0	0.0			2
<i>Mansonia perturbans</i>	24	0	27	1	0	0	3.7		1	1

* Based on all late dissections and including those with advanced larvae as positive.

† Index: 1—Important economic species; 2—Locally abundant and annoying, principally out of doors; 3—Common species, not very troublesome; 4—Usually rare or of very restricted distribution (Based on King, Bradley, and McNeel, 1944).

Table 3 compares susceptibility data for this species from studies on infections of different origin. The first double column refers to recent work on the nocturnal variety, whereas the last two double columns are referable to the type which has been known as the "non-periodic."

Investigations of the nocturnal strain by Newton *et al.* (1945) showed that *C. quinquefasciatus* was almost completely susceptible (this is in accord with the

work of previous investigators as mentioned in the introduction). The work of Bahr (1912) on the other hand showed that a smaller percentage of *C. quinquefasciatus* (as *C. fatigans*) were infected when fed on the Fiji (Pacific Island) strain. In addition, where Newton *et al.*, found an average of over 6 late stage larvae per specimen, Bahr found only 1. In the work reported here, substantial agreement with Bahr's data was secured but the mean number of advanced larvae was found to average 2.7 per specimen.

The lots tested in this study were typical *C. quinquefasciatus* Say from coastal South Carolina. The identity of these lots is demonstrated by two facts: (1) A sizeable number of terminalia were examined from one of the coastal stations; (2) These stations are all south of the range of *C. pipiens*. Recent extensive

TABLE 3
Comparison of susceptibility of mosquitoes to *W. bancrofti* of different origin
Results of three investigations

SPECIES	PUERTO RICO STRAIN* (NOCTURNAL)		SOCIETY ISLANDS STRAIN†		FIJI STRAIN‡	
	Number dissected	Per cent showing late forms	Number dissected	Per cent showing late forms	Number dissected§	Per cent showing late forms
<i>Culex quinquefasciatus</i>	17	100.0	106	34.9	15	26.7-33.3
<i>Aedes aegypti</i>	102	4.9	90	3.3	28	0.0
<i>Aedes triseriatus</i>	107	2.8	120	6.7		
<i>Anopheles punctipennis</i>	95	0.0	118	2.5		
<i>Anopheles quadrimaculatus</i>	10	0.0	120	0.0		
<i>Culex erraticus</i>	8	0.0	35	22.9		

* Newton *et al.*, 1945.

† Present Study.

‡ Bahr, 1912.

§ Bahr's data have been summarized here as to dissections after 10 days to conform with data from Newton *et al.*, 1945, and the present study.

|| In the case of some *C. quinquefasciatus* dissections, it is impossible to tell from the description whether or not the larvae were normal.

collections by the U. S. Army have failed to demonstrate the presence of the latter species in the general region.

An additional lot of *Culex* collected at Columbia, S. C., was fed. It was initially assumed that these were *C. quinquefasciatus* as this species predominates over *C. pipiens* in this locality, but no terminalia were examined. Susceptibility was in all respects similar to *C. pipiens* both as to percentage infected and the intensity of infection (table 2). Examination of Army data discloses that *C. pipiens* occurs with *C. quinquefasciatus* in Columbia with *C. quinquefasciatus* 250 times as frequent. Further data will be necessary to find out if this lot represents (1) *C. pipiens*, (2) a strain of *C. quinquefasciatus* differing from the coastal *C. quinquefasciatus* in susceptibility to *W. bancrofti*, or (3) an intergradation between the two species.

A point which would favor both *C. quinquefasciatus* and *C. pipiens* in the trans-

mission of disease is their comparative long life. In insectary experience the great majority of the individuals of lots of this species can be kept alive for 15 to 20 days or longer, but in other species, especially the *Anopheles*, greater mortality was noted. These remarks are made with the realization that such insectary data are not necessarily applicable to field conditions.

Culex erraticus D. and K. and *C. salinarius* Coq. are also long-lived mosquitoes but are not habitually found in close association with man, although the former is sometimes abundant especially in the vicinity of permanent, *Lemna* covered bodies of water and the latter is widely distributed and often bites man.

In the case of *C. erraticus*, 22.9 per cent were found to bring the infection near or to the infective stage, whereas in *C. salinarius* 2.9 per cent were found with advanced larvae but none with infective larvae in the proboscis. In the light of the above it is unlikely that these species are of potential menace. Comparing in table 3, it will be noted that of 8 dissected Newton *et al.* (1945) found none infected (personal communication indicated that later work showed a small percentage to be susceptible), but due to the small number dissected by them, this fact is not out of accord with our data.

Of the *Anopheles* tested, *A. walkeri* Theo. gave the heaviest infection but the data are of limited value owing to the fact that only a small number were dissected. It was very difficult to maintain the species alive in the insectary, and only 14 individuals lived over 10 days. Of these, 5 or 35.7 per cent had advanced larvae; only two had infective stage larvae, and these were not in the proboscis. Attesting, however, to the validity of the data from late dissections is the fact that of 36 mosquitoes dissected before the 10th day, 12 or 33.3 per cent showed young larvae which appeared to be developing normally. It might be noted here that in most cases in which development was aborted, the abortion occurred during the early part of the first stage.

Despite the apparent moderate infection rate of this species, it could hardly become an effective vector due to its irregular, discontinuous distribution, and its apparent short life.

Anopheles punctipennis (Say) is very widely distributed and frequently bites man but seldom attains a great adult density; therefore, the light infection rate of this species would indicate that it would not be an important vector of *W. bancrofti* in this country.

During the early development of *W. bancrofti* in this host worms penetrate the thorax and shorten somewhat in most instances, but development usually ceases at what might be called the early sausage stage. Aborted first stage forms are often found in the thorax even after 15 days, the non-developing forms being in some cases active and in others degenerated and apparently dead.

Comparison of the data presented here with those of Newton *et al.* does not indicate a significant difference (1.4 standard deviations) between the response of *A. punctipennis* to the two strains of *W. bancrofti*.

Aedes triseriatus (Say) is a widely distributed tree-hole breeder, and although a frequent and an annoying biter is seldom present in great abundance. Considering the low susceptibility (6.7 per cent) and the low intensity of infection,

it seems extremely unlikely that this species could efficiently transmit *W. bancrofti* in this country. As in *Anopheles punctipennis*, aborted first stage larvae were found frequently in the thorax in this species after as much as 15 days.

Newton *et al.* found only 2.8 per cent infected in a comparable large lot of *A. triseriatus*. The difference between the two strains does not appear to be significant.

The domesticity of *Aedes aegypti* (L.) would make it an important potential vector of *W. bancrofti* were it not for the fortunate fact that it is only slightly susceptible to infection with this strain of the filaria. Even though only 3.3 per cent of a large lot was found infected, its close relationship to man would indicate that it could not be disregarded. The results of Newton *et al.* working with the nocturnal strain are essentially similar to ours (4.9 per cent), while Bahr's negative results (table 3) may be due to the small number dissected.

Single infected specimens of *Aedes atropalpus* (Coq.) and *Mansonia perturbans* (Walk.) were found in small lots fed on *W. bancrofti*. In the case of the former, which is rare and of restricted distribution, only one of 7 was found to have infective larvae (in thorax, but not in head or proboscis). In the case of *M. perturbans* a somewhat larger sample was handled and the authors consider that the data are indicative of very low susceptibility especially as the dissection of 24 additional specimens before the 10th day showed no cases of normal development. In the single case in which advanced development was found, the single larva had progressed only to a late third stage. It might, therefore, be concluded that despite the local abundance of *M. perturbans* it is unlikely that it represents a potentially efficient vector.

SUMMARY AND CONCLUSIONS

Susceptibility experiments with a strain of *Wuchereria bancrofti* (Cobbold) from Bora Bora in the Society Islands showed that development to advanced or infective stages occurred in the following United States mosquitoes: *Culex pipiens*, *C. quinquefasciatus*, *C. erraticus*, *C. salinarius*, *Anopheles walkeri*, *A. punctipennis*, *Aedes triseriatus*, *A. aegypti*, *A. atropalpus*, and *Mansonia perturbans*. Taking into consideration the habits and prevalence of the species, it was concluded that only *Culex pipiens* (83.5 per cent infected) and possibly *Culex quinquefasciatus* (34.9 per cent infected) were sufficiently susceptible to be dangerous potential vectors.

Anopheles quadrimaculatus, *A. freeborni*, *Aedes atlanticus* (or *tormentor*), and *Psorophora ferox* failed to develop the infection beyond the early first larval stage.

The authors gratefully acknowledge the aid of the staff at Moore General Hospital, the late Colonel Frank W. Wilson, Commanding, where this work was done as a part of a cooperative study between the U. S. Public Health Service and the United States Army. Especial thanks are due the patient who voluntarily and unselfishly fed several thousand mosquitoes, thus putting himself to considerable personal inconvenience.

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USE OF DIMETHYLPHTHALATE IMPREGNATED CLOTHING AS PROTECTION AGAINST SCRUB TYPHUS

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Although laboratory and field tests have indicated that dimethylphthalate will prevent the attachment of itch-producing mites, there are no reports in the literature actually demonstrating the efficacy of this compound in preventing scrub typhus in the field.

An opportunity presented itself on an island in the South Pacific, to study the protection afforded by the application of dimethylphthalate by two different techniques as compared with the lack of any protection, during the months from August to November, 1944. During this period the equivalent of four and a half infantry battalions operated in the Y River area (see figure 1). For the purpose of this analysis this group can be divided into three units: first, one unit (1 battalion in strength) which used no miticide-treated clothing; second, a unit (1 battalion in strength) which had all clothing sprayed with dimethylphthalate, and third, a unit (2½ battalions in strength) which had all uniforms impregnated with an emulsion of 5 per cent dimethylphthalate as described in War Department Technical Bulletin, TB Med 121, December, 1944. The last unit was the largest of the group.

The technique for preparing the emulsion of dimethylphthalate and the actual impregnation of clothing is quite simple, and is accomplished readily in the field with facilities at hand. To prepare enough emulsion to impregnate 100 uniforms, 6 pounds of laundry soap is cut into small pieces and dissolved in 10 gallons of water by boiling in an oil drum. To this is added 25 gallons of cold water and heating is discontinued. Three gallons of this soap solution is poured into a can and 7½ quarts of dimethylphthalate is slowly added and the solution is vigorously whipped to make a creamy concentrate. This concentrate is now returned to the drum of soap solution and stirred to make the finished emulsion. This final product is a 5 per cent emulsion of dimethylphthalate in a 2 per cent soap solution, measuring about 37 gallons. The clothes, with sox secured in pockets, are completely immersed in this solution and then wrung out and hung up to dry. The clothing is ready to wear when dry. Untreated shorts must be worn with this clothing to prevent scrotal irritation.

On 9 August 1944, the 1st Battalion of X Infantry Regiment began operations in the Y River area on the Island of Z. A few days after their return within the perimeter, on 23 August 1944, cases of scrub typhus began to appear among the personnel of this battalion. The 2nd Battalion of X Infantry Regiment followed the 1st Battalion.

With reference to figure 1, troops of the 1st and 2nd Battalions were deployed at locations marked Area 1 and 2 and along trails marked 3, 4, 5, and 6. X represents the mouth of Y River. The 2nd Battalion was replaced by the 3rd

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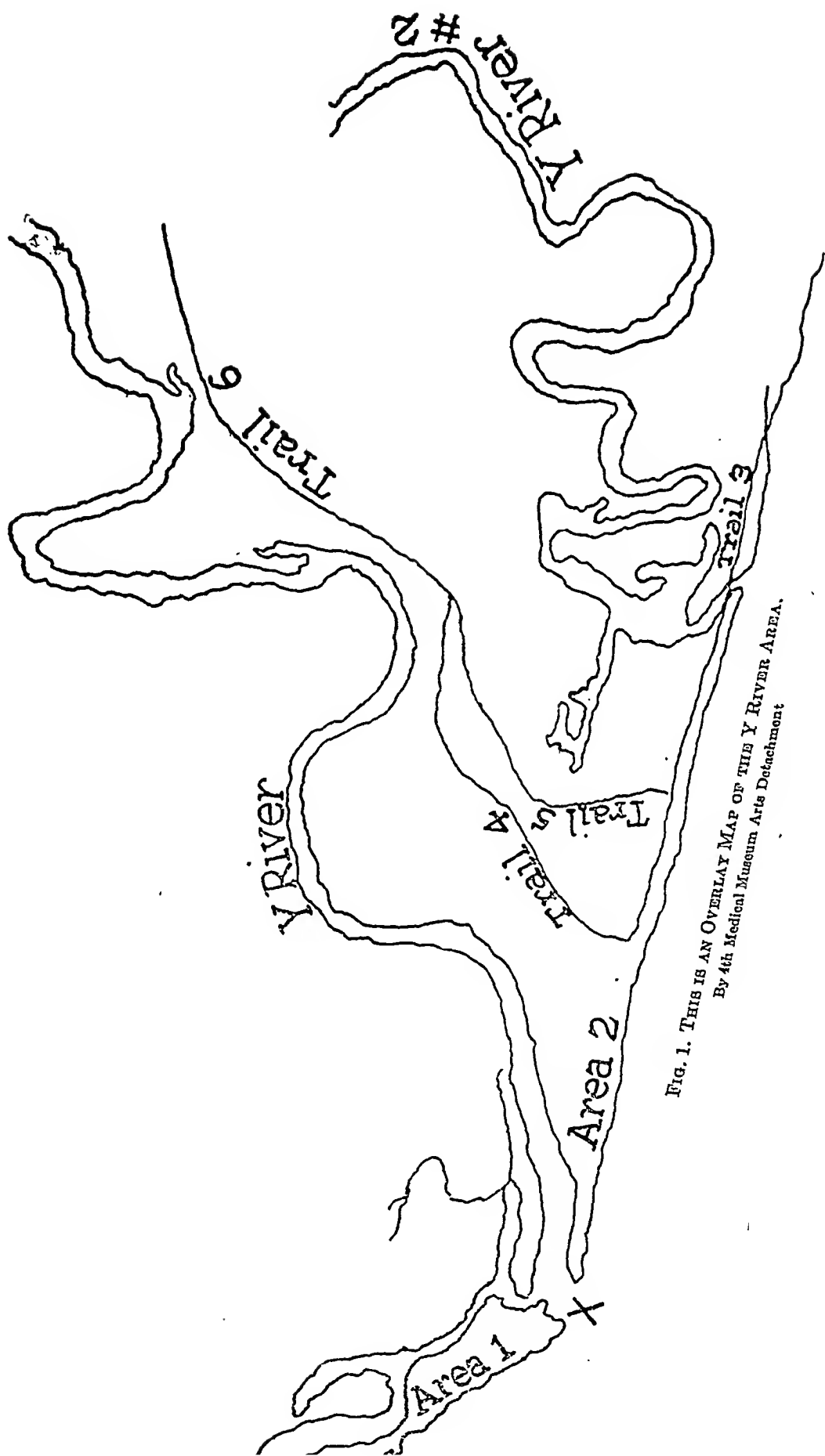


FIG. 1. THIS IS AN OVERLAY MAP OF THE Y RIVER AREA.
By 4th Medical Museum Arts Detachment

Battalion and, following them, troops in re-inforced company strength from this and other regiments of the division were used in this combat problem for periods of 7 to 10 days at a time. The 3rd Battalion and successive troops, representing the equivalent of two and a half battalions in strength in all, were deployed for the most part at Area 1 and a few troops in small numbers operated at Area 2.

Although it is impossible to state the locations of all the foci of infection in the region represented by figure 1, the evidence is clear that Area 1 was certainly one, if not the only one, of these. The evidence indicting Area 1 as a focus of infection is as follows:

1. Troops from A Company of 1st Battalion who had very few cases of scrub typhus had the *least* contact with Area 1.

2. Questioning of troops with the disease revealed that some had only been in Area 1 and had never been across the Y River in Area 2 or along any of the trails.

3. Area 1 was investigated by members of the Navy Medical Research Unit #2. They found rats in profusion, and from these obtained *Trombicula*

TABLE 1

GROUP	MITICIDE	AREA OF OPERATION	DURATION OF OPERATION	NUMBER OF CASES
1st Battalion	None	Area 1 and 2, trails 3, 4, 5 and 6.	7-10 days	45
2nd Battalion	Clothes sprayed with dimethylphthalate	Area 1 and 2, trails 3, 4, 5 and 6.	7-10 days	16
3rd Battalion and successive troops. (2½ battalions)	Clothes impregnated with dimethylphthalate	Area 1 and a few troops in Area 2.	7-10 days	7

deliensis mites which have been suspected as vectors of scrub typhus. For tactical reasons these investigators were unable to explore any of the areas across the Y River.

Troops of the 1st Battalion used no specific precautions to protect themselves from mites, beyond the casual use of standard mosquito repellent on exposed surfaces of the skin at night. The repellent used in most cases was not dimethylphthalate.

Troops of the 2nd Battalion had their clothes sprayed with dimethylphthalate by hand operated paint spray-gun. However, not all troops were so processed.

Beginning with the 3rd Battalion and all subsequent troops operating in this area, all clothes worn were impregnated with a 5 per cent emulsion of dimethylphthalate as previously described. In regard to this last group, it must be added that many of them slept on cots in their fox-holes, rather than on the ground, which was usually the case with troops of the 1st and 2nd Battalions.

Table 1 is a summary of the varying protections afforded these three groups and the resulting incidence of scrub typhus.

Unfortunately the precise strengths of these three groups are not available. However, it is fair to assume that each battalion was approximately equal in strength. Thus, the third group which was two and a half battalions in strength had an incidence rate of one fifteenth that of the 1st Battalion and one fifth that of the 2nd Battalion.

It could be argued that this was not a critical test of the efficiency of dimethylphthalate impregnated clothing against scrub typhus because troops other than those from 1st and 2nd Battalions had not been deployed in all the areas. Although this is the case, it is considered that the risk of infection with scrub typhus was at least equal in all the three units, since it has been shown that Area 1 was actually an endemic scrub typhus area. Another factor that must be taken into consideration is the use of cots for sleeping by personnel of the third group. It is well known that sleeping directly on the ground enhances the opportunity for attachment by mites; therefore, this factor may have played a role in the reduction of infection. On the other hand, the third group had much less patrol activity than the 1st and 2nd Battalions and had more stationary contact with the ground in Area 1 during the day. It should be mentioned that there were no instances of dermatitis among troops wearing the impregnated clothing.

SUMMARY

The incidence of scrub typhus in three groups of troops operating in an area where one of the probable vectors of this disease was found and where various degrees of protection against the mite was used has been analyzed. Troops with no protection had the highest incidence of the disease, as compared with a lower incidence in troops where clothes were sprayed with dimethylphthalate, and lowest in the group whose clothes had been actually impregnated with a 5 per cent emulsion of dimethylphthalate. There were no instances of dermatitis.

It is considered that this experience offers evidence indicative of the high degree of protection against scrub typhus provided by clothes impregnated with dimethylphthalate.

SHIGELLA CARRIERS WITH SPECIAL REFERENCE TO THEIR THERAPY, INCLUDING THE USE OF STREPTOMYCIN^{1, 2}

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Four outbreaks of shigellosis recently occurred aboard a Naval vessel (1). Several shigella carriers were found who were considered to be the source of infection in the last two outbreaks. A study of these carriers (with particular reference to their therapy) was undertaken since they constituted a major problem in eradication of the disease.

It is obvious that a large number of persistent shigella carriers can be an epidemiological problem. Manson-Bahr (2) reports an individual who excreted dysentery bacilli for a period of three years. In an excellent review, Neter (3) states that carrier rate surveys have varied from 3-80 per cent and that 3 per cent of convalescent carriers excrete organisms for more than three months after the onset of the disease. Watt *et al.* (4) studied 57 cases of *Shigella flexneri* infections. Among this group 45 individuals (79 per cent) became convalescent carriers, in 38 of whom the duration of the carrier state was determined. At the end of a 10 week period, four (11 per cent) were still carriers. The authors do not record the duration of the carrier state in these four cases, although they conclude that shigella carriers of over one year duration are of little epidemiological importance. However, these four relatively persistent carriers represent about 7 per cent of the original group of flexner infections studied. Perry (5) found that the carrier state persisted for at least one year in 7 per cent of the cases of infections with the flexner type. If one can assume, on this basis, that during an extensive outbreak due to *Shigella flexneri*, approximately 7 per cent of the cases will become relatively persistent carriers, then the carrier problem cannot be eliminated from epidemiological considerations.

Furthermore, Manson-Bahr (2) has pointed out that the majority of apparently healthy carriers are still suffering from ulceration of the intestinal mucosa. Felsen (6) also reports the frequent finding of intestinal lesions in supposedly well carriers.

There are numerous encouraging reports regarding the effectiveness of sulfonamides in eliminating carrier states (7). However, Sandweiss (8) found sulfathalidine ineffective in the therapy of *Shigella* carriers in the Detroit area. Some

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success has been reported with bacteriophage (9). Nevertheless, Boyd and Portnoy's (10) conclusion that bacteriophage is relatively ineffective *in vivo* probably represents an accurate appraisal of the present status of bacteriophage therapy.

DESCRIPTION OF CARRIER GROUP

The circumstances of four outbreaks which resulted in a group of 72 carriers have already been reported (1). Many of the men studied had had attacks of enteritis from one to five months previously, though a few had never had any such symptoms. Every carrier was asymptomatic at the time of this study, and all were kept isolated in a hospital ward, facilitating close observation. Several of the men were sigmoidoscoped. A thickened injected rectal mucosa was noted in only one man.

Prior to inclusion in this study, 38 of these men had been given 31 grams of sulfadiazine each over a seven day period. These 38 failures with sulfadiazine represented approximately 60 per cent of the original group treated, indicating that sulfadiazine in the dosage employed was not effective for the treatment of these carriers.

This study was conducted over a period of four and a half months. During this time a total of 1,278 cultures were made while these men were hospitalized.

BACTERIOLOGICAL METHODS AND OBSERVATIONS

All follow-up specimens were obtained by the rectal swab technic, and plated on salmonella-shigella agar. All suspicious colonies were put into Kligler's iron agar medium and all organisms showing typical shigella reactions were confirmed by agglutination in type-specific antisera. Every carrier harbored an identical organism, a strain of *Shigella flexneri* type III, possessing a secondary antigen which agglutinated type VIII sera. An organism with similar serological characteristics has been isolated from an epidemic of bacillary dysentery in Leyte Gulf (12) and we have isolated the same organism from the personnel of two other ships returning from the Orient.

Sulfadiazine resistance studies were done on strains isolated from six of these carriers. As controls, five stock cultures of various *Shigella* types were also checked. A sulfonamide inhibitor-free broth medium was used which was shown to support the growth of shigella organisms readily. It was clearly demonstrated that the *Shigella flexneri* III (VIII) organism we were dealing with in these carriers was highly resistant to sulfadiazine. Table 1 summarizes the data on these experiments. These results confirm the observations of Cheever (12).

In vitro tests of sensitivity of this organism to penicillin and streptomycin were also completed. As expected, no sensitivity to large amounts of penicillin (50 units per cc.) was demonstrated; however, a marked *in vitro* sensitivity to streptomycin was observed. Nutrient broth (Difco), adjusted to a pH of 8 was a very satisfactory medium for checking streptomycin resistance, and was used in these tests. Seven colonies of *Shigella flexneri* III (VIII) isolated from six carriers were tested; four of the colonies had been recovered from carriers before

TABLE 1
Sulfadiazine resistance tests*

SULFADIAZINE CONCENTRATION	CARRIER STRAIN S. FLEXNERI III (VIII)					
	#1	#2	#3	#4	#5	#6
<i>mgm. per cent</i>						
100	++++	++++	++++	++++	++++	++++
50	++++	++++	++++	++++	++++	++++
25	++++	++++	++++	++++	++++	++++
12.5	++++	++++	++++	++++	++++	++++
6.25	++++	++++	++++	++++	++++	++++
3.12	++++	++++	++++	++++	++++	++++
1.56	++++	++++	++++	++++	++++	++++
0.78	++++	++++	++++	++++	++++	++++
0	++++	++++	++++	++++	++++	++++

	STOCK CULTURES SHIGELLA				
	<i>flexneri III</i>	<i>flexneri VIII</i>	<i>flexneri II</i>	<i>dysenteriae</i>	<i>sonnei</i>
100	0	0	0	0	0
50	0	0	0	0	0
25	0	0	0	0	0
12.5	0	0	0	0	0
6.25	0	0	0	0	0
3.12	0	0	++	++	0
1.56	0	0	++++	++++	++++
.78	0	++	++++	++++	++++
0	++++	++++	++++	++++	++++

* Similar results were obtained with sulfathiazole but sulfanilamide was less effective against the stock cultures.

0 equals complete inhibition of growth.

++++ equals no inhibition of growth.

TABLE 2
Streptomycin sensitivity tests with S. flexneri III (VIII)

STREPTOMYCIN CONCENTRATION, UNITS PER CC.	COLONIES ISOLATED BEFORE STREPTOMYCIN THERAPY				COLONIES ISOLATED AFTER STREPTOMYCIN THERAPY		
	Patient #1	Patient #2	Patient #3	Patient #4	Patient #1	Patient #5	Patient #6
6.25	0	0	0	0	0	0	0
3.12	0	0	0	0	0	0	0
1.56	0	0	0	0	0	0	0
0.78	0	0	0	0	0	0	0
0.39	0	0	0	0	0	0	0
0.20	0	0	0	0	0	0	0
0.10	0	++++	++++	++++	++++	++++	++++
0.05	++++	++++	++++	++++	++++	++++	++++

streptomycin therapy was begun and three after it had been completed. The results are indicated in table 2. From these data we judged that no streptomycin fastness developed as a result of therapy.

DESCRIPTION OF THERAPY PLANS

This relatively large number of segregated carriers, all harboring the same organism, constituted an excellent group for the evaluation of therapy. Initially, 67 of these carriers were divided arbitrarily into the following six groups:

A. Controls; 12 men given placebo only.

B. Succinylsulfathiazole; 10 men given three grams every four hours for seven days.

C. Penicillin; 11 men given 50,000 units intramuscularly every three hours for seven days.

D. Succinylsulfathiazole; 12 men given three grams every four hours for seven days, plus oral penicillin (200,000 units initially followed by 50,000 units every two hours) for the last four days.

E. Sulfathalidine; 12 men given three grams every four hours for seven days.

F. *S. Flexneri* III (VIII) vaccine in a concentration of 500 million organisms per cc. 10 men given seven injections (0.1, 0.2, 0.4, 0.8, 1.0, 1.0, and 1.0 cc. respectively, at 3 day intervals over a 21 day period.

Individuals in groups A through E all had positive rectal cultures within a few days of the initiation of therapy, and can be considered comparable. Nine in Group F, however, had their last positive rectal cultures as long as two weeks before the onset of therapy, and probably some of these individuals had cleared spontaneously before therapy was begun. Four other proven persistent carriers, who had been treatment failures in one or more of the other therapy plans, were subsequently added to Group F.

Later in this study a limited amount of streptomycin was available for evaluation. Only oral therapy was employed. The dosage was 125,000 units every three hours over a five day period, or a total dosage of 5,000,000 units (5 grams of streptomycin base) per case. Only known persistent carriers, who excreted organisms for over a two months period and had been treatment failures with other therapy, were included in this, Group G.

Bacteriophage⁶ was also given to five known persistent carriers, Group H. The phage employed produced complete lysis of an 18 hour culture through a dilution of 1×10^{-8} . These five carriers received 10 cc. of phage by mouth daily for five days. It is apparent that groups G and H cannot be regarded as strictly comparable to the other six groups, since both consist entirely of persistent carriers.

METHODS OF EVALUATION AND RESULTS OF THERAPY

It was possible to follow all carriers for a minimum of five consecutive negative rectal cultures, taken over a five week's period, since no carrier was discharged from the hospital until this criterion was fulfilled. In addition, it was possible to follow about 50 per cent of these carriers by rectal culture after hospital discharge. In the case of "persistent" carriers a minimum of 12 consecutive negative rectal cultures over a five week period was required before discharge from the hospital.

⁶ Obtained from Overly Biochemical Research Foundation, Inc., New York;

In one instance 10 consecutive negative rectal cultures were obtained over a two month period before another positive culture was found. In even persistent carriers less than 50 per cent of their cultures were positive, indicating the intermittency with which carriers excrete organisms.

Table 3 records results of therapy in terms of the carrier state, one and two months after therapy was started. A negative carrier state indicates that all rectal cultures taken during the month and subsequently (a minimum of 5 consecutive cultures *in toto*) were negative for *Shigella flexneri* III (VIII). No complications were encountered as a result of any therapy.

TABLE 3
Results of therapy of *S. flexneri* III (VIII) carriers

GROUP	THERAPY PLAN	TOTAL NO. CARRIERS IN GROUP	CARRIER STATE 1 MONTH AFTER ONSET OF THERAPY		CARRIER STATE† TWO MONTHS AFTER ONSET OF THERAPY	
			Negative*	Positive	Negative*	Positive
A	Control	12	8	4	2	2
B	Succinylsulfathiazole	10	7	3	1	2
C	Penicillin, i.m.	11	7	4	1	3
D	Succinylsulfathiazole plus oral penicillin	12	10	2	1	1
E	Sulfathalidine	12	7	5†	1	3
F	<i>S. flexneri</i> III (VIII) Vaccine	14	11	3	0	3
G	Streptomycin	10	7	3	2	1
H	Bacteriophage	5	0	5	Not observed	

* A negative carrier state is one in which all rectal cultures taken during the month and all subsequent cultures (minimum of 5 consecutive cultures) were negative for *S. flexneri* III (VIII).

† Only individuals tabulated are men regarded as persistent carriers up to one month after onset of therapy.

‡ One man not followed for second month.

OBSERVATIONS OF CARRIERS RECEIVING STREPTOMYCIN

As expected, no streptomycin was demonstrated in the blood of one carrier on whom blood assays were done.⁷ Stool streptomycin assays were done using 1 per cent peptone agar and employing a modified cup plate method (13). As much as 85 per cent of the streptomycin was recovered from the stools.

The bacterial flora of the stools from 10 carriers was also followed by daily rectal cultures plated on eosin-methylene-blue medium. It was quite apparent that the total mean growth was markedly reduced while these carriers were receiving streptomycin therapy. An average growth of approximately 200 colonies per plate was found in the pre- and post-streptomycin periods, whereas an average of less than 100 colonies per plate was found during streptomycin therapy.

⁷ Done by S. F. Quon, Hooper Research Foundation, University of California.

COMMENT

Eleven men (16 per cent) of the original 67 carriers studied excreted organisms for longer than a two months period (*i.e.* were "persistent" carriers by our definition). Convalescent carrier rates in *Shigella flexneri*, infections of 79 per cent have been reported (4). If these carrier rates are significant, then we can assume that 12 per cent of all patients with *Shigella flexneri* infections become persistent carriers. These rates would tend to point to the importance of recognition and isolation of carriers in the control of recurrent outbreaks of dysentery.

A statistical analysis of our results (Table III) would not be valid because the numbers are small and the groups are not comparable. As mentioned previously only persistent carriers who had been treatment failures with one or more of the other plans were given streptomycin. Undoubtedly the results with streptomycin therapy would have appeared more encouraging if carriers had been placed in this group by random selection, as in the case of groups A to E. Of four similar persistent carriers given vaccine, only one had cleared in the second month after therapy was started. Bacteriophage failed in the five additional persistent carriers. The organism that these carriers harbored was shown to be highly sensitive to streptomycin. The cessation of regularly positive rectal cultures was abrupt in several carriers during or immediately after streptomycin therapy. We feel that these results were promising and warrant the further trial of streptomycin for persistent shigella carriers, despite our failure to produce cures in all instances. Possibly larger doses and the combination of oral and systemic streptomycin therapy would be more efficacious.

The strain of *Shigella flexneri* III (VIII) was highly resistant to sulfadiazine, in contrast to the susceptibility of several other shigella organisms tested. It was to be expected then that neither the absorbable or poorly absorbable sulfonamides would be effective in eliminating the carrier state in these individuals.

The intermittency with which positive rectal cultures were secured from these carriers was notable. Probably many reported cures result from failure to obtain a sufficient number of cultures. We feel that in the case of shigella carriers, at least 12 consecutive negative cultures taken over a reasonable time interval should be required before a bacteriological cure can be assumed.

CONCLUSIONS

1. Persistent shigella carriers can become an important epidemiological consideration in dealing with control measures for shigellosis.
2. The strain of *Shigella flexneri* III (VIII) isolated from these carriers has been shown to be highly resistant to the bacteriostatic action of sulfadiazine; however, it was very sensitive to the action of streptomycin *in vitro*.
3. Streptomycin seemed to offer some promise, and we feel that further trial with this drug in treatment of persistent shigella carriers is warranted.
4. The intermittency with which positive cultures are obtained from carriers makes it difficult to establish a bacteriological cure in these individuals. Prob-

ably twelve (12) consecutive negative cultures is a fairly adequate criterion for cure.

We wish to express our thanks to Capt. E. F. Evans, (MC) USN for his co-operation in this study.

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THE CUTICULAR MORPHOLOGY OF SOME COMMON MICROFILARIAE

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A review of the literature on the morphology of microfilariae, with special reference to the cuticula, leaves one in doubt about the presence or absence of transverse striations. Selected illustrations indicate the divergent opinions that exist.

Fülleborn (1) summarizes as follows, "The cuticula of microfilariae possesses a distinct cross-girdling which, in stained preparations, often gives the organism the appearance of an earthworm. According to Bahr, on the non-periodic *bancrofti* larvae of the southseas the cross-striation begins 6μ from the anterior end of the parasite. Rodenwaldt also reports them to begin about 6 to 10μ from the anterior end of the dog microfilaria. In both species the striations can be followed to the utmost tip of the tail.

"These striations sometimes appear as deep dark red lines if the organism is vitally stained with azure stain followed by eosin differentiation. There are, however, two longitudinal bands, one on each side of the parasite, where the azure-eosin striations are absent. As these bands are on the sides of the microfilaria they may be called the 'side lines' of the worm. Where the azure-eosin striations end at the bright band a small dark knob is found on each striation. Meinhof has also seen these knobs when the worm was stained weakly with hematoxylin. One can sometimes observe such tiny grain-like deposits where the cuticula is interrupted by a body opening. Thus they occur at the margin of the excretory pore and, according to Rodenwaldt, who first described these marginal deposits, they also appear near the position of the tail flexure." Fülleborn illustrates *Mf. bancrofti* with transverse striations in the shrunken form but without them in the non-shrunken form. Neither of 2 photomicrographs of the *Mf.* stained with Azure II after hot alcohol fixation reveals striations.

Manson-Bahr (2) says, "A closely set, very delicate transverse striation can be detected in the musculo-cutaneous layer throughout the entire length of the embryo," and his artist's drawing shows *Mf. bancrofti* with delicate transverse striations. An original drawing of a *W. bancrofti* larva in a mosquito's thorax shows well marked transverse striations along its entire length. Strong (3) states for *Mf. bancrofti*, "The cuticula is smooth," whereas Rivas (4) states, "The body is transversely striated." Hegner, Root, Augustine, and Huff (5) present no textual description but an illustration after Feng depicts *Mf. bancrofti* with indications of transverse striations at the lateral margins only. Faust (6) writes, "The cuticula (of *Mf. bancrofti*) is usually described as having delicate transverse striations but these are probably artifacts due to fixation." Fantham, Stephens, and Theobald (7) describe it as follows, "Cuticle: Transversely striated. There is a longitudinal break in the striations on each side corresponding to the

lateral lines. The striation is best shown by vital staining with Azure II and eosin differentiation." This description may have been based upon actual observations but the wording suggests that it was taken from Fülleborn. Their illustrations, after Fülleborn, do not show striations even in the shrunken form. It seems apparent that van der Sar and Hartz (8), confronted with the transverse striations that show so well in their photomicrographs, hesitated to say that the *Mf.* in lymph nodes from patients with tropical eosinophilia were *Mf. bancrofti*, but contented themselves by saying, "We did not succeed in determining the species of filariae found in our cases, but up till now only *Wuchereria bancrofti* has been found on Curaçao."

With regard to *Mf. loa* the situation is less ambiguous. Fülleborn stated that the cuticula is transversely striated, and reproduced photomicrographs that showed them. His artist depicted them in one plate but not in another. On the other hand Braun (9) stated, "The cuticle is smooth." The illustration selected by Fantham, Stephens, and Theobald, from Fülleborn, is the one that does not show striations. Manson-Bahr illustrates the transverse striations but does not mention them in the text. In his description of *Mf. papionis* Treadgold (10) writes, "The cuticle is transversely striated, and its appearance closely corresponds to Fülleborn's diagram of the cuticle of *Mf. loa*." After comparing the *Mf.* of *Filaria striata* Molin, 1858, with those of *Filariopsis asper*, van Thiel (11) says of the former, "The microfilariae are ensheathed and 228 to 255 μ long, the cuticle being finely striated transversely. . . . The whole of the microfilaria much resembles those of *Filaria bancrofti* and *Loa loa*, and differs markedly from that of *Filariopsis asper*."

Faust described *Mf. malayi* by saying, "The cuticula is very delicately striated," and this appears to be the accepted view. Faust and Braun are in agreement that *Mf. magalhãesi* possesses delicate transverse striations. Here, again, no dissenting opinions were found. Although *Mf. ozzardi* and *Mf. perstans* are striated, according to Fülleborn, only in the shrunken forms Fantham, Stephens, and Theobald say that the cuticula of *Mf. perstans* is transversely striated. Most other authors do not describe the cuticula. Although most texts do not describe the cuticula of *Mf. immitis* there is in MacQueen's revision of Neumann's text (12) an illustration, after Railliet, that shows transverse striations on this species. I have not found reference to the presence or absence of cuticular striations on *Mf. carinii*. By means of a modified Hortega stain beautifully clear, annular, transverse striations were demonstrated on *Mf. volvulus* by German (13).

It was assumed that these incomplete and often contradictory descriptions of the cuticula implied that transverse striations were undoubtedly present on some species, and possibly on most or all; that the usually recommended stains might fail to reveal them, and that the factors of time, temperature, anticoagulant, or dehemoglobinizing agents used to prepare smears of *Mf.* in blood might induce alterations of, or degenerative changes in, the cuticula. Such changes might render existing striations invisible by any or all methods of staining. These assumptions were tested as follows.

MATERIALS

Blood smears containing *Mf.* of available species were collected from different sources. Some had been dehemoglobinized and stained with hematoxylin, with and without eosin differentiation; others were dehemoglobinized but unstained, and the remainder were freshly prepared, unlaked smears.

METHOD OF STAINING

Since German obtained excellent staining of cuticular structures of *Mf.* in tissues by means of a silver impregnation technique it seemed that one of the flagellar stains that involve silver deposition might stain these structures on *Mf.* in blood smears. The Saisawa-Sugawara stain was selected as one of the best of these methods. It was used to stain both unstained and previously stained blood smears that contained *Mf.* This excellent stain has not received the attention it deserves. Since it is published only in Japanese literature, and with slightly variable formulas, it is given below in the form that gave best results here.

Saisawa-Sugawara stain

Solution A.

Tannic acid.....	5.0
Distilled water to	100.0

Dissolve with minimal heat. When cool, filter and add in the following order:

Fe_2Cl_6 , 40% aqueous solution.....	1.5
Formalin.....	2.0
NaOH, 10% aqueous solution.....	1.5

Shake well. It should have an indigo color. The whiter the tannic acid in color, the better the mordant.

Solution B.

AgNO_3	2.0
Distilled water to	100.0

Dissolve. Take 10.0 ml into a beaker. While shaking, add strong ammonia water drop by drop until opalescence appears and disappears. Then add the remaining 90 ml and shake well. It should show a brownish turbidity.

If kept separately in well stoppered brown bottles these solutions will remain useful for at least a month.

PROCEDURE FOR STAINING BLOOD SMEARS

A small amount of each well shaken solution was filtered through paper just before smears were to be stained. For smears previously stained with hematoxylin the area was flooded with filtrate of solution A for from 5 to 8 minutes, the duration depending upon the depth of mordanting desired. The mordant was flooded off with a stream of distilled water and the slide was then washed in a dish in running tap water for 4 or 5 minutes. The smear was again flushed with distilled water and, with the wet slide held over a white surface, was flooded quickly with the filtrate of solution B, and the slide tilted back and forth over a white surface until it developed an evenly distributed deep orange brown color. This required from 3 to 20 seconds. As soon as the desired color developed the solu-

tion was quickly washed off with distilled water, and the slide again washed for several minutes in running tap water, dried in air, and mounted in clarite.

When the technique was applied to dried dehemoglobinized smears the embryos were usually shrunken. Shrinkage was largely avoided by hydrating the smears for 15 to 30 minutes in water and allowing them to just become dry in air before mordanting and staining. Unlaked smears were dehemoglobinized in distilled water, or water containing low concentrations of acetic acid and formalin. If they were mordanted and stained while still wet the silver penetrated the cuticula and stained the nuclei. If smears were mordanted shortly after they dried in air the silver was deposited upon the cuticula.

MORPHOLOGY OF THE CUTICULA IN SILVER STAINED SMEARS

If the smears had been stained previously with hematoxylin the nuclei were visible and, encircling them, there was a series of annular transverse striations that extended from the tip of the head to the tip of the tail. Cuticular striations were well shown on some specimens that were stained here or elsewhere with hematoxylin, with and without eosin differentiation. However, they were visible on the cephalic tip in only a small number. If the eye held the focal plane at or about the level of the nuclei the striations were rarely visible on the first 5 to 10 μ of the cephalic end. Furthermore, since the apparent density of this end is greater than that of the remainder of the embryo, striations could be seen there only by means of critical illumination, appropriate color filters, extreme care in focusing, and sometimes only by oblique illumination. It is not surprising that they have not been seen regularly in this location in preparations stained with hematoxylin. After silver staining they were readily apparent throughout the entire lengths of the embryos. They were usually seen better under oil immersion than with a high dry objective, and better with a Wratten B filter, or a combination of B and E filters, than with white light. Either the B or H filter was best for photography. Mf. that were not shrunken by preparatory procedures showed the striations best.

By means of the silver stain striations were demonstrated on Mf. of the following species: *W. bancrofti*, *W. malayi*, *L. loa*, *M. ozzardi*, *A. perstans*, *O. volvulus*, *D. immitis*, and *L. carinii*. The striations are spaced regularly at the same interval in all species. Many measurements along straight or straighter portions gave counts that varied only between 12 and 13 striations to each 11.11 μ . Hence the average distance between striations is about 0.9 μ .

Striations were demonstrated readily on the Mf. of all species except *L. carinii*. With other species, after alcohol-ether fixation, the mordant and silver never penetrated the cuticula unless the mordant was applied before the smear was completely dry. In contrast, the silver always penetrated the cuticula and stained the cell nuclei of Mf. *carinii* in dried smears that were fixed with alcohol-ether, and often did so if no fixative other than drying was used. It may be that the cuticula of this species differs in composition from those of the other species studied. Although only 5 or 6 embryos out of many hundreds showed regularly spaced striations it is believed that the cuticula is striated but it is recognized

that the demonstration rests on far less secure grounds with respect to *L. carinii* than to the other species.

A study of many Mf., well stained by either hematoxylin or the Saisawa-Sugawara stain, failed to reveal the longitudinal bands that have been variously called the white, lateral, or side lines. If nuclear columns were stained, thereby practically compelling the eye to adopt the focal plane of the nuclei rather than that of the rounded surface of the cuticula, simulacra of side lines were readily obtained. Under these conditions the terminal knobs on the striations that were described by Rodenwaldt and Meinhof, and further described and diagrammed by Fülleborn, also became apparent. They appeared along both margins of the side lines at the points where these lines cut each transverse striation. However, critical examination of many such Mf., always oriented in the same position with reference to the cephalic end and nerve ring, revealed that these side lines always remained in the same location, parallel to and about $1\ \mu$ within the lateral margins of the cuticula, even though the excretory pore was now at the left lateral margin, now at the superior surface, and again at the right lateral margin or underneath the embryo. This fact in itself, that the side lines remain at the lateral margins while embryos are rotated on their longitudinal axes through 360 degrees, demonstrates conclusively that the alleged side lines are not anatomical structures but that they exist only in the eye of the observer. Furthermore, a gradual change of focus from just beneath the level of the nuclei to the level of the nuclear plane and, finally, to the level of the striations on the rounded superior surface of the cuticula demonstrated clearly that the longitudinal white lines and the knobby ends of the transverse striations were optical artifacts caused by a transection of the arching transverse striations by the focal plane. Elevation of the focus by as little as $1\ \mu$ was sufficient to dispel these images, and under no other conditions could they be visualized.

In smears that were silvered without previous nuclear staining the Mf. showed only the annular transverse striations upon the cuticular surfaces. Nuclei and all subcuticular structures were invisible in such preparations. Hence the striations are not only present at the extreme cephalic tips but they are actually on the cuticula, not in the musculo-cutaneous layer. Furthermore, no embryo of any species stained in this manner revealed either a longitudinal or side line, or any knobs on the transverse striations.

Since German recently illustrated so beautifully the striations on Mf. of *O. volvulus* space is not taken here to show them in slide preparations. The few obtainable specimens of Mf. of *W. malayi* had been prepared by a method that almost obliterated the finer cuticular details. One Mf. showed striations extending to the cephalic tip after silver staining but none were visible in the hematoxylin stained slides. Since all authorities agree that this species is striated the rather poor specimens examined here are not reproduced. Although there is general agreement that the Mf. of *Loa loa* are striated, and although Fülleborn's photomicrographs are wholly convincing, none of about 500 embryos examined here revealed a complete series of striations. Only where an end, or a portion of a loop, was covered by coagulated plasma could short striated sections

be seen. Here, again, the method of preparation had resulted in almost complete obliteration of all superficial structural details. About 100 slides of *Mf. bancrofti* had been prepared by the standard method in the same laboratory that supplied the *Mf. loa*. Careful restaining and critical examination of many of these slides showed that of numerous *Mf.* examined only a rare one revealed a few striations, in marked contrast to the results obtained with other lots of *Mf. bancrofti* that were prepared by the same method.

Although it is unlikely that all causes for degenerative changes in the cuticula have been found, limited observations have incriminated bacterial contamination of dehemoglobinizing waters as one of the chief factors. All slides that were poor from the viewpoint of cuticular morphology were supplied by a laboratory within the tropics where slides were being prepared in large numbers for teaching purposes. Every smear showed numerous bacteria and spores. The smears that showed normal cuticulae, some also prepared within the tropics, had been completed shortly after blood was taken from patients by physicians eager to establish the diagnosis quickly. None of these slides showed either bacteria or spores. There is less convincing evidence that an unduly long exposure of smears to laking solutions may also induce degenerative changes. Both difficulties are easily controllable by the addition of small quantities of acetic acid and formaldehyde to dehemoglobinizing waters, using the least amount necessary to shorten the laking time and to inhibit microbial growth. I have added 4 cc. of a 1 per cent solution of acetic acid in 5 per cent formalin (containing about 38 per cent of formaldehyde) to about 60 cc. of water in slide jars. Most human blood smears were well laked in 15 minutes, but some required about $\frac{1}{2}$ hour. Smears of rat blood required longer exposures. The agent that causes degenerative changes in the cuticula of *Mf.* also causes degeneration of the cytoplasm of leukocytes. Consequently a good indication of what may be expected from silver staining can be had by examining the leukocytes in a hematoxylin stained smear. Whenever leukocytes show a normal amount of cytoplasm striations can be demonstrated. But if leukocytes are markedly deficient in cytoplasm, with marginal indentations between compressed nuclear lobes, no method of staining can be expected to reveal cuticular striations throughout the lengths of the embryos. I wish to emphasize that, severe as these superficial degenerative changes have been, they have never interfered with correct species diagnosis of the various *Mf.* in hematoxylin stained smears. This easily demonstrated fact, together with cuticular degeneration inflicted during dehemoglobinization, probably due to microbial contamination, best explains the past experiences that have led to the existing uncertainties with respect to the presence or absence of cuticular striations. If *Mf.* are to be prepared to reveal their cuticular striations care must be taken to apply the least damaging preparatory procedure for the minimal effective time.

SUMMARY

By means of the Saisawa-Sugawara silver deposition method it was demonstrated that the commoner blood and tissue microfilariae possess annular transverse cuticular striations that completely cover the embryos from tip to tip.

Striated cuticulae were demonstrated on the Mf. of *Wuchereria bancrofti*, *W. malayi*, *Loa loa*, *Mansonella ozzardi*, *Acanthocheilonema perstans*, *Onchocerca volvulus*, *Dirofilaria immitis* and, with less certainty, on *Litomosoides carinii*.

No embryo of the 8 species examined possessed a lateral or side line. These alleged lines, and the knobby ends of the striations, were demonstrated to be optical artifacts.

Acknowledgment: For supplies of blood smears gratitude is expressed to Lt. Comdr. J. C. Riffe, (MC), USNR, Dr. A. D. Welch, Dr. F. J. Brady, Dr. J. T. Culbertson, and to Maj. G. W. Hunter, III, and Capt. L. S. West, and their associates in the Division of Parasitology, Army Medical School.

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PLATE I

Photomicrographs of microfilariae in blood smears that were restained with the Saisawa-Sugawara silver stain after initial staining with hematoxylin.

Upper left. *W. bancrofti*. The evenly spaced annular transverse cuticular striations are not visible on the cephalic tip at this focus which shows simulacra of a longitudinal line and knobs on the striations at the upper margin of the cephalic one-sixth of the embryo. Initial magnification 720 \times .

Upper right. *W. bancrofti*. The focus on the lower coils of the unshrunk embryo coincides with the rounded superior surface of the cuticula to show the annular envelopment of the columns of nuclei by the striated cuticula. Initial magnification 1350 \times .

Middle right. *M. ozzardi*. Cephalic end in focus to show striations extending to the extreme tip. Initial magnification 1350 \times .

Middle left. *W. bancrofti*. Cephalic end of an embryo showing striations to the extreme tip. Initial magnification 1350 \times .

Lower left. *W. bancrofti*. The encircling striations are visible to the extreme cephalic tip. Initial magnification 1350 \times .

Lower right. *A. perstans*. Although this embryo was embedded in plasma coagulum its striations are visible from tip to tip. Initial magnification 1350 \times .

PLATE I

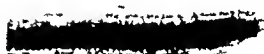


PLATE II

Photomicrographs of microfilariae in blood smears stained by the silver deposition method without initial nuclear staining. Initial magnifications 1350 \times . Note that subcuticular structures are not visible; also the lack of evidence for the existence of longitudinal lines and knobby striations.

Upper left. *W. bancrofti*. Striations are scarcely visible at the cephalic tip which is somewhat shrunken. The small objects out of focus are bacteria and spores. The remainder of this embryo showed moderately advanced cuticular degenerative changes.

Upper right. *D. immitis*. Silvered lightly to reveal cuticular details.

Lower left. *L. carinii*. One of the best photomicrographs obtained with this species, taken with vertical light. The faintly visible cross lines occur at the same interval as do the striations of other species, and probably represent true cuticular striations.

Lower right. *D. immitis*. Silvered heavily and photographed with a Wratten No. 45 H filter in an unsuccessful effort to disclose longitudinal lines or interruptions in the striations.

PLATE II



TISSUE PATHOLOGY OF EXPERIMENTAL TSUTSUGAMUSHI DISEASE (SCRUB TYPHUS) IN SWISS MICE AND MACACUS RHEBUS MONKEYS AND THE REPORT OF ONE HUMAN CASE ACQUIRED IN THE LABORATORY*

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INTRODUCTION

Farner and Katsampes (1) dismiss the subject of the pathology of tsutsugamushi disease with the single statement, "The same basic micropathology of vasculitis and perivasculitis has been described in postmortem material from Japan, Malaya, New Guinea, Formosa, and Australia." Ashburn and Craig (2) in 1908 state that knowledge of the pathologic anatomy of tsutsugamushi was incomplete because, "in the region of flood fever . . . there is a sentiment against autopsies". In 1943, Forbus (3) held the same thing to be true. Ahlm and Lipshutz (4) in reporting human cases stressed only the clinical and serological features. The outstanding contribution to the pathology of the disease was made by Kawamura in his monograph in 1926 (5). He published the gross and microscopic observations on seven human necropsies, one ape and nine monkeys. The gross pathology in twelve human cases and the microscopic pathology in seven human brains were described by Lewthwaite in 1936 (6). The gross and microscopic pathology in humans was presented by Corbett (seven cases) in 1943 (7), and by Lipman et al (six cases) in 1944 (8). Allen and Spitz (9) and Browning et al (10) have subsequently added materially to our understanding of the disease.

The work reported below was undertaken in order to study the pathology of experimentally induced tsutsugamushi disease (scrub typhus) in Swiss mice and *Macacus rhesus* monkeys. The human case discussed serves as a basis of comparison for the tissue changes present in the advanced stage of the disease in animals and also, adds an additional case to the rapidly expanding literature.

METHODS AND MATERIALS

Eighty-seven mice, ten monkeys, and one human case form the basis of this study. The standard dose of egg yolk suspension of the Karp strain⁶ of *Ricket-*

* The material in this article should be construed only as the personal opinion of the writers and not as representing the opinion of the Navy Department officially.

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⁶ Validity as a true agent of the disease demonstrated by McLimans et al (11).

tsia orientalis for mice which allowed a ten day mean survival time was established at 1000 M.L.D. when injected intraperitoneally. Twenty-five mice so treated, with sixteen normal controls, were killed at intervals and studied for progressive pathological changes. Sixteen of the infected mice and ten of the controls were injected with 0.1 cc. of India ink intravenously one and/or two hours before autopsy to determine the role of macrophages in the disease and to accentuate the phenomenon of what McLimans et al (11) call "endothelial stickiness" (leukocyte adherence to the endothelial lining of blood and lymph vessels).

A separate series of twenty mice was inoculated with 1000 M.L.D. Karp strain intraperitoneally and two infected and one control were killed the same hour each day following inoculation. These animals were used to study progressive lymphoid changes in the spleen and lymph nodes.

The monkeys were inoculated intradermally, intravenously and intraperitoneally with an egg yolk suspension or with an infected guinea pig spleen suspension of the virus. Of the ten monkeys inoculated, five completely recovered and were not killed. One of the remaining five died accidentally on the fourteenth day post inoculation and was not autopsied. The other four were killed on the eleventh, twelfth, twenty-third and twenty-eighth day post inoculation.

The mice were killed with illuminating gas and the monkeys with intravenous sodium pentothal. Immediately after death all the animals were perfused through the heart with normal saline solution followed by fixation with formol-Zenker solution for twenty-four hours, washed twenty-four hours, imbedded in celloidin, sectioned at twelve micra and stained with hematoxylin-eosin-azure. The technic used in preparation of the slides and the terminology used in description of the cells closely follow the methods and terminology of Maximow and Bloom. HEA stain of celloidin sections which emphasized individual cell characteristics facilitated the study of the hematological-mesenchymal cellular response to the disease. The stain coupled with the selective phagocytosis of injected ink by the macrophages made cell identification easy and accurate.

OBSERVATIONS

1. *Swiss mice; the acute and terminal stages of the disease.*—The day by day organ changes in tsutsugamushi disease were studied in mice in an effort to determine the pathological sequence. The lymphoid organs signaled the changes that later involved all tissues. The lymph nodes in the normal animals frequently contained some degree of hyperplasia manifested by diffuseness of the cortex with loss of follicular structure. Likewise, the amount of mitotic activity varied indiscriminantly in both diseased and "normal" nodes. We, therefore, decided not to use lymph nodes as a basis of comparison for the various stages of the disease, but to confine such observations to the more consistent changes in the spleen. The spleen is the first organ to show pathologic changes in Swiss mice infected with *Rickettsia orientalis*. The general systemic involvement lags one to two days behind the spleen involvement.

In the controls the microscopic appearance of the spleen is fairly uniform.

The chief features of normal spleen morphology are: (a) follicles averaging 38.8 micra in diameter, (b) quiescent perivascular white pulp composed chiefly of small lymphocytes and containing only rare mitotic cells, and (c) abundant normal-appearing red pulp. The follicular (Malpighian corpuscle) structure consists of a lymphocytopoietic center containing large reticular cells, large lymphocytes, a few macrophages as demonstrated in the ink-injected series, and a few small lymphocytes. Surrounding these centers, which show only moderate-to-mild mitotic activity, there is a collar of small lymphocytes. This collar acts as a sharp border for the follicle causing it to stand out in marked contrast to the surrounding pulp.

On the second or third day after inoculation, there is usually an increased mitotic activity in the center of the follicles.

Until the fourth day, the process is chiefly one of general hyperplasia, but from then on, even though hyperplasia of the reticular cells continues for a time in the lymphopoietic centers, the over-all number of cells in the follicle decreases. The loss takes place in the lymphocytic collar which becomes progressively thinner and less cellular. As the cells migrate from the periphery of the follicles, the average diameter of the follicles decreases.

By the sixth and seventh days the lymphocytic collar around the centers is thin and blends into the surrounding pulp. Mitotic figures continue in moderate numbers in the center of the follicles and are also found in the perivascular lymphocytic collections in the white pulp. These latter are increased in size and number.

On the eighth and ninth days, mitotic figures and small lymphocytes in the centers of the follicles are less common and the lymphocytic collars are often not perceptible. The lack of sharp borders to the follicles plus the increased amount and diffuse nature of the white pulp is so marked by the ninth day that accurate measurement of the diameter of the follicles becomes in many cases impossible.

By the tenth day the follicles are composed of large reticular cells with only a few large and small lymphocytes and only a few mitotic figures. The collars of adult lymphocytes around the follicles are absent. The perivascular white pulp is diffuse and contains but few mitotic figures.

The average diameters of the Malpighian corpuscles of seventeen infected and ten control mice are plotted (fig. 1). Two features are of particular interest: (a) the steady trend of decreasing size of the corpuscles of the infected mice and (b) the difference in size of the corpuscles during the early or subclinical stage of the disease (first four days post inoculation) when the size of the corpuscles of seven of the eight spleens is within the size range of the controls and during the active or clinical stage of the disease (the last six days) when, coinciding with the reported (11) blood lymphocytosis and the generalized tissue invasion, the size of the corpuscles is below the range and is much smaller than the average of the controls. Assuming that the size of the corpuscles of the controls and of the infected mice differs by chance alone (null hypothesis) the probability that the means of the controls should differ from the means of the infected mice by the

amount exhibited by the data is of the order of one or two chances in ten during the first four days ($t = 1.91$, $P = 0.2-0.1$), while during the last six days it is of the order of less than one chance in 10,000 ($t = 6.69$, $P \ll 0.01$). Thus, it may be very safely concluded that no significant differentiation in size of corpuscles of the spleen is possible in the first period, but highly significant differentiation is possible in the last period.

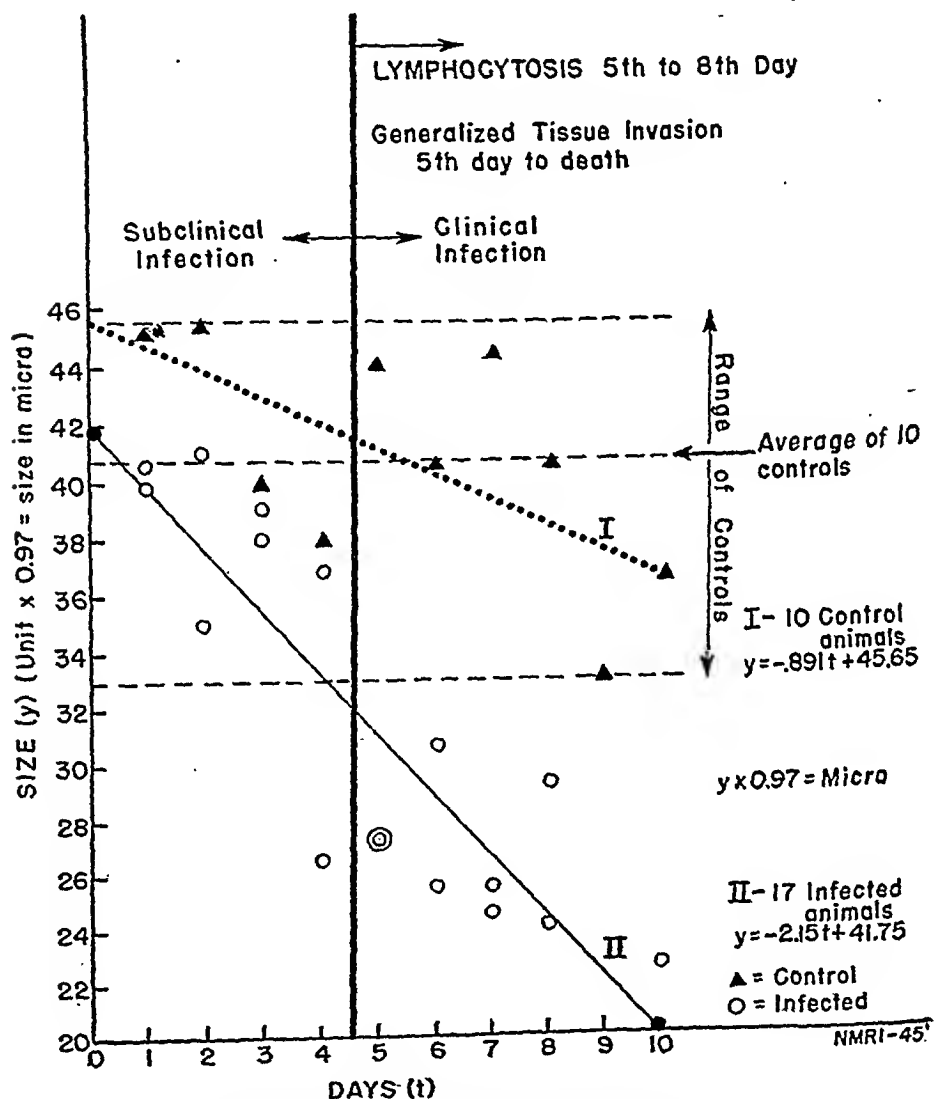


FIG. 1. THE AVERAGE DIAMETER OF MALPIGHIAN CORPUSCLES OF THE SPLEENS OF CONTROLS AND INFECTED MICE

The ink-injected series proves that in all stages of the disease the phagocytic cells are located chiefly outside the follicles in the white pulp. The greatest concentrations of ink-containing cells are found at the outer margin of the lymphoid collars of the follicle, but others are scattered through the pulp and are found occasionally in the follicle centers.

Histologic details of tissues other than the spleen follow.

Heart (Plate 2, fig. 11).—There is a generalized lymphoid-macrophage infiltration beginning about the fifth day and increasing with the duration of the disease. It is not a specific lesion but resembles chronic myocarditis. The cells congregate around the blood vessels, and, in the terminal stages of the disease, numerous plasma cells are present. All layers of the heart are involved.

Lungs (Plate 2, fig. 15).—Beginning about the sixth or seventh day the alveolar walls become progressively thickened and moderately infiltrated with lymphocytes. In the terminal stages, "endothelial stickiness" is prominent with numerous pigment-containing macrophages adhering to the vessel walls. Perivascular collections of lymphocytes and macrophages and a few plasma cells are present after the eighth day. The alveoli themselves remain clear and air filled. The bronchioles sometimes show slight edema of the connective tissue and slight lymphocytic infiltration. The mucosa appears normal.

Gastro-intestinal Tract (Plate 4, fig. 31).—Lymphocytes, macrophages, and plasma cells are increased in the lamina propria of the stomach, small intestine and colon, beginning about the sixth or seventh day post inoculation and becoming increasingly prominent until death. In addition, there is a milder response in the tunica muscularis. The serosal surface often contains small lymphoid collections and the mesothelium is swollen. The submucosa and the epithelium appear normal although occasionally a small perivascular cellular reaction may be seen in the submucosa.

Liver (Plate 3, fig. 19).—The liver does not show much change until about the eighth day post inoculation when macrophages and lymphocytes become adherent to the endothelium along with a mild periportal lymphocytic infiltration. In some of the livers, particularly in those from animals in the late stages of the disease, a slight perivascular collection of lymphocytes appears around the central veins. Some Kupffer cells are swollen but there is no evidence of their proliferation. The liver cells are frequently vacuolated. Occasionally where large collections of lymphocytes and macrophages fill and apparently block sinusoids, the neighboring liver cells are necrotic. The capsule of the liver in many of the animals contains an exudate heavily infiltrated with lymphocytes and occasionally with polymorphonuclear leucocytes.

Pancreas.—By the fifth day there is a generalized mild lymphoid infiltration which becomes more prominent as the disease progresses. In the late stages, a few perivascular collections of lymphocytes and macrophages are present. Slight "endothelial stickiness" is also seen.

Spleen (Plate 1, figs. 1-10; Plate 5, figs. 39-42; Plate 6, fig. 43).—Described in detail above and in plate legends.

Adrenal Gland.—Small clumps of lymphocytes are found in the interstitial tissue in cortex of some of the glands. The peri-adrenal fat sometimes contains an increased number of lymphocytes.

Kidneys (Plate 3, fig. 23).—The same interstitial changes and "endothelial stickiness" seen in the other organs are found in the kidney. The glomerular and tubular epithelia are not damaged. No casts are present in the renal tubules.

The Lymph Nodes.—The majority of the cells are small lymphocytes with a scattering of large lymphocytes and plasma cells. The sinusoids of the lymph

nodes are filled with macrophages particularly in the late stages of the disease, and the follicles usually reach a state of marked cellular depletion with greatly decreased mitotic activity. Necrotic areas are not seen.

Striated muscle.—In many animals the muscle sections appear normal, but in others they contain mild generalized and/or perivascular infiltration of lymphocytes and macrophages. Occasional mast cells are seen in the connective tissue around the joints.

Skin.—Changes in the skin are very slight. The epithelium and subcutaneous tissue appear normal. The dermis occasionally contains a few small collections of lymphocytes in perivascular areas.

Brain (Plate 4, fig. 27).—All parts of the mid-brain and cerebellum appear normal. Occasionally a small focus of lymphocytes is seen in the substance of the medulla and in the cerebral cortex. The most striking changes occur in the pia-arachnoid where small perivascular lymphoid-macrophage collections are sometimes found.

The bone marrow appears normal.

2. *Monkeys: the convalescent stage of the disease.*—Our observations on the recovery or repair stage of this disease are based on the study of two monkeys killed on the twenty-third and twenty-eighth days post inoculation. They had been quite sick, but when killed had been convalescent for nine to ten days. Two monkeys that had been running a fever up to 105–106°F. for eight and seven days previously were killed on the eleventh and twelfth days post inoculation. The microscopic findings in these severely ill monkeys are similar to the findings in mice in the late stages of the disease.

Skin ulcers (Plate 5, figs. 35, 36) which formed at the site of inoculation were excised on the eighth day and on the twelfth day post inoculation. Both show ulceration and slight undermining of the epithelium with invasion of the dermis. The necrotic ulcer floor in the dermis shows heavy infiltration with acute and chronic inflammatory cells. In the earlier biopsy there is marked edema of the deeper layers of the dermis while in the later one granulation tissue is present in the subcutaneous layer. The limits of the inflammatory process are not well defined in either biopsy, but spread laterally and gradually fade into normal tissue. Biopsy of an inguinal lymph node (Plate 5, figs. 37, 38) removed on the tenth day post inoculation from one of the monkeys which recovered, shows marked hypercellularity with a predominance of macrophages. The sinuses are distended and filled with macrophages, lymphocytes and plasma cells. The lymph follicles are diffuse. There is no perivascular reaction and no necrosis.

The tissues of monkeys in the repair or convalescent stage of the disease show changes similar to but more marked than the changes seen in the acute stage of the disease as described in mice. As the disease process ages, the lymphocyte-macrophage infiltration of the various organs becomes more pronounced; the perivascular collections of cells become larger and denser; adherence of lymphoid-macrophage cells to the vascular endothelium in the perfused specimens becomes less prominent and less common, and finally, small areas of cell necrosis appear, particularly in the vicinity of the larger perivascular collections of cells.

The morphological findings described below in detail refer to the monkeys killed on the twenty-third and twenty-eighth day and show the condition of the organs in the repair stage of the disease.

Heart (Plate 2, figs. 12, 14).—There is a generalized and focal perivascular lymphocyte and plasma cell infiltration through the stroma of the myocardium. Perivascular collections of cells are fairly common in the subepicardial and the subendocardial regions.

Lungs (Plate 2, figs. 16, 18).—The alveoli are clear, but the walls are slightly hypercellular due to a mild lymphocyte-monocyte infiltration. There is mild perivascular collaring. The bronchioles appear normal.

Gastro-intestinal Tract (Plate 4, figs. 32, 34).—Some of the vessels of the submucosa and muscularis of the *stomach* show perivascular lymphocyte collections and the lamina propria contains lymphocytes and plasma cells with a few polymorphonuclears. In the *small intestine* perivascular lymphocytes are prominent in the submucosa, and large masses of macrophages containing unidentified inclusions are present in the sub-epithelial spaces of the lamina propria. The *large intestine* is characterized by dense plasma cell collections in the lamina propria.

Liver (Plate 3, figs. 20, 22).—The liver cells are within normal limits except in regions where large collections of lymphocytes, plasma cells and macrophages are associated with necrosis of a few neighboring liver cells. These necrotic areas are usually in or near the portal spaces. In both the central vein and portal areas, lymphocytes and macrophages form pronounced perivascular collars. Often in these cell collections, large cells with large pale nuclei and much deeply basophilic cytoplasm, presumably hemocytoblasts, are found. Some of the macrophages contain inclusions which cannot be clearly defined. The capsule of the liver appears unaltered.

Pancreas.—Lymphocytes and plasma cells in the ratio of about two to one are found in a few small collections scattered through the stroma.

Spleen (Plate 6, figs. 44, 46).—The malpighian corpuscles are distinct with normal germinal centers. Plasma cells are abundant in the red pulp and the endothelium of the small vessels appears normal.

Adrenal gland.—The adrenal gland appears normal.

Kidneys (Plate 3, figs. 24, 26).—Focal interstitial areas contain lymphocytes and plasma cells, occasionally monocytes and rare polymorphonuclears which appear both in the cortex and in the medulla. The tubular and glomerular epithelia appear normal. Some of the proximal convoluted tubules contain amorphous, granular, eosin-staining material.

Lymph Nodes.—The lymph follicles are fairly discrete and seem slightly enlarged though not hyperplastic. The sinuses contain markedly increased numbers of macrophages with cellular debris and large lymphocytes. In the smaller vessels in the loose areolar tissue around the lymph node, a slight "endothelial stickiness" is present.

Striated muscle.—The muscle appears normal except for a few small foci of lymphocytes in the fascia between muscle bundles.

Skin.—The epidermis, dermis, abdominal muscularis appear normal. The

subcutaneous tissue shows moderate numbers of lymphocytes. The peritoneum is slightly thickened due to a mild lymphocytic infiltration.

Brain (Plate 4, figs. 28, 30).—A few focal collections of lymphocytes are present in the substance of the brain stem and in the cerebrum. The pia-arachnoid and choroid plexus appear normal and no nerve cell damage is apparent.

Submaxillary Glands.—A few collections of lymphocytes, plasma cells, and macrophages in areas near small vessels and a slight diffuse plasma cell infiltration of the gland are seen.

Pituitary, Thyroid, Eye and Bone Marrow appear normal.

3. *Man: the terminal stage of the disease.*—Tissue blocks were obtained from a 31 year old man who contracted tsutsugamushi disease while working with yolk sac material of *Rickettsia orientalis*. This case is unusual because although the portal by which the disease gained access to the body is unknown it is assumed to have been via the respiratory tract. The patient could not recall any accident in the laboratory involving contact of the virus with the skin, nor was he working with the mite vector. At no time in the course of his illness was a skin ulceration or eschar seen. The presence of the ulceration is an important clinical diagnostic point of tsutsugamushi disease in humans. It is present in the great majority of field-acquired cases and there is always some doubt cast on the diagnosis when no eschar is demonstrable. However, in this case the disease agent was demonstrated by contact smears made of the spleen at the time of autopsy. The smears contained numerous intracellular bodies with the staining and morphological characteristics of rickettsia. In addition, portions of spleen ground up and injected into mice and monkeys caused these animals to develop clinical signs and pathologic tissue changes characteristic of tsutsugamushi disease.

On admission to the hospital⁷ the patient gave a history of headache, chilly sensations, and general muscular aches of two days duration. He also complained of anorexia and temperature varying from 99.8 to 101.6°F. He had not noted any body rash. Several years previously the patient had recovered from an infection of European typhus. His temperature at time of the present admission was 100.8°F., blood pressure, 130/76. Heart and lungs were negative. There was no palpable lymphadenopathy and his skin was clear. Complete blood chemistry studies were within normal limits. Sedimentation rate was 20 mm. in one hour. Agglutination tests for *Proteus* OX-K and OX-19 were negative. Kahn test was negative. Admission blood count and urinalysis were not remarkable.

A chest x-ray taken the day after admission was reported negative, but when repeated with a portable film three days later it showed parahilar and basal pneumonia bilaterally. This picture persisted until death. Electrocardiogram on admission was negative, but during the course of the disease abnormalities developed consisting of minor changes of "T" waves in amplitude and shape together with an arrhythmia due to fairly regular paroxysms of 4-5 auricular premature beats.

⁷ U. S. Naval Hospital, National Naval Medical Center, Bethesda, Md.

Throughout his hospital stay, the temperature of the patient fluctuated between 100 and 104°F. The day after admission a very transitory maculopapular rash developed in the axillae and on the chest. The patient became apprehensive. Periods of confusion and lethargy ensued but there were no localizing neurologic signs. On the twelfth hospital day Proteus OX-K titer rose to 1 to 320; OX-19 agglutinations remained negative. In addition to the usual supportive therapy, the patient received 750 cc. of plasma from three donors who had recovered from naturally occurring scrub typhus six to nine months previously. He was also given para-amino-benzoic acid, eight grams, as an initial dose prior to admission and two grams every two hours day and night up to the day before death. There was no significant alteration in his blood studies. His course was progressively downhill and he died from respiratory failure fifteen days after admission and seventeen days after the onset of illness.

The autopsy was begun one hour and forty minutes after death. Thin blocks of tissue were fixed for twenty-four hours in formol-Zenker solution and were treated thereafter in the same manner as the tissue of the experimental animals. The abnormal findings noted at autopsy were bilateral pleural effusion, bilateral moist heavy lungs with multiple nodular areas of consolidation, firm slightly enlarged pancreas and slight edema of the brain. A few of the mesenteric lymph nodes were thought to be slightly enlarged but the majority appeared normal. Liver and spleen appeared normal.

The tissue reaction in this case is, in general, comparable with the late invasive stage seen in the experimental animals. The same focal and diffuse lymphocyte-macrophage-plasma cell infiltrations are seen in nearly all the organs studied. Perivascular cell collections and focal necroses are about as prominent as those seen in the experimental animals in the late stage of the disease. Features present in the human case, but not seen in the experimental animals include pulmonary edema and congestion with early interstitial pneumonia (often called "virus" pneumonia). The histological changes are reported in detail.

Heart (Plate 2, fig. 18).—There is a generalized diffuse and focal lymphocyte-plasma cell-macrophage infiltration of the interstitial connective tissue, and occasional polymorphonuclear leucocytes and mast cells are seen. All three layers of the heart are involved. The cells collect in the perivascular spaces and create a collaring effect. The macrophages often contain engulfed material, some of which can be identified as white cell nuclei.

Lungs (Plate 2, fig. 17).—In some regions alveolar spaces are filled with pale eosin-staining edema fluid; in others, they are filled with macrophages, lymphocytes or red cells. A few alveoli around the bronchioles contain polymorphonuclear leucocytes and occasional strands of fibrin. The alveolar walls throughout are thickened and infiltrated with lymphocytes, plasma cells and a few polymorphonuclears. The alveolar capillaries and the epithelium of the bronchioles appear normal, but the loose connective tissue around the bronchioles contains a few lymphocytes and plasma cells. The lumina of the bronchioles often contain edema fluid and scattered cells.

Gastro-Intestinal Tract (Plate 4, fig. 33).—Marked changes occur in the *small intestine* which shows a fairly heavy sprinkling of lymphocytes, plasma cells and macrophages in the lamina propria. A few polymorphonuclears are also seen in this location. The same types of cells are present in the perivascular spaces in the tunica muscularis. The vascular endothelium appears normal. The *stomach* and *colon* show similar but less marked changes. The epithelium appears normal throughout the gastro-intestinal tract.

Liver (Plate 3, fig. 21).—Small lymphocytic collections in some of the portal areas, vacuolated parenchymatous cells, and congested sinusoids around some of the central veins are the only abnormalities noted.

Pancreas (Plate 6, fig. 49).—Focal and diffuse interstitial infiltration particularly around blood vessels involving lymphocytes, plasma cells, and polymorphonuclears is the only abnormal finding.

Spleen (Plate 6, fig. 45).—The sinuses are congested and packed with macrophages. Although the basic architectural pattern of the spleen is maintained, the follicles are small, pale, and partially depleted of cells. They blend into the surrounding pulp and suggest a general picture similar to that seen in the terminal stages of the disease in the spleens of mice. Mitotic activity is low. The vascular endothelium appears normal.

Adrenal gland.—The parenchyma appears normal, but the surrounding fatty connective tissue contains a marked concentration of macrophages, lymphocytes and plasma cells and a few mast cells.

Kidneys (Plate 3, fig. 25; Plate 6, fig. 48).—The interstitial tissue of both cortex and medulla contains lymphocytes, macrophages, and plasma cells in both diffuse and focal collections. Except in a few subcapsular areas where there are wedge-shaped scars, the glomeruli appear normal. In the scarred areas these are replaced by fibrous tissue containing plasma cells and lymphocytes. Most tubules appear normal, but occasionally one is found with irregular homogeneous or coarsely granular material in the lumen. The vascular endothelium appears normal, but circulating macrophages are abundant. In one segment of the lumen of a venule, 26 macrophages are found to the exclusion of all other cells. In the subepithelial tissue particularly in the perivascular spaces around the calyces, lymphocytes and plasma cells are abundant.

Lymph Nodes (Plate 6, fig. 50).—The follicles are poorly defined and appear diffuse and exhausted. The germinal centers consist of reticular cells and macrophages containing inclusions. The sinuses are filled with macrophages, many of which are vacuolated and contain inclusions, plasma cells and lymphocytes. Mitotic activity is at a low ebb.

Striated Muscle (including diaphragm).—Most sections appear normal, but a few show a very mild interstitial infiltration of lymphocytes and plasma cells.

Skin.—A very slight tendency toward perivascular collaring is seen.

Brain (and spinal cord).—Sections through many levels show no abnormalities. In the substance of the mid-brain a rare collection of lymphocytes is found, but in the pia-arachnoid foci of lymphocytes are fairly common.

Thyroid appears normal.

DISCUSSION

All animals studied in our experiments were inoculated with the Karp strain of *Rickettsia orientalis* and after an incubation period of four to five days clinical signs of illness became increasingly apparent. The animals became listless and lethargic, they developed anorexia, and assumed unnatural resting attitudes in their cages. Their blood became infective on and after the fourth day. Terminally, there was some respiratory distress and frequently abdominal swelling. The mice died on the ninth to twelfth day but the monkeys gradually recovered after about fifteen days of illness. In addition to observations of the clinical course, as a further check on the fact that we were dealing with tsutsugamushi disease, we made impression smears of the spleen routinely and of other organs occasionally. Air-dried and stained for thirty minutes with Giemsa, then washed for one minute with distilled water, buffered to a pH of 6.8, these smears when examined at a magnification of 900 times or better showed typical bi-polar intracellular rickettsia. Our numerous attempts using various fixatives and stains have failed to produce organ sections satisfactory for demonstration of the rickettsia in fixed tissue. Lewthwaite (6) was able to demonstrate rickettsia in the perivascular macrophages of five of the seven brains he studied. More recent authors do not mention an attempt at tissue staining for the rickettsia of tsutsugamushi disease. For diagnosis we have relied on organ impression smears, the characteristic pathological picture, the transmissibility of the disease by blood passage and the curve of the Proteus OX-K agglutination titers.

Our findings in Swiss mice and *Macacus rhesus* monkeys and one human generally confirm those of other observers (5, 6, 7, 8, 9, 10, 11). Swiss mice react to inoculations of yolk sac suspensions of the rickettsia with 99 per cent mortality by the ninth to twelfth day post inoculation. The course of the disease allows too little time for degenerative changes to take place with the result that widespread focal necroses stressed as a feature of the disease by the above named authors are rarely found in the mice. In monkeys and man, where the disease pursues a more leisurely course, (none of our monkeys succumbed to the disease while the human case lived for seventeen days after the onset of his illness), small focal necroses in the various organs are found with more regularity. The basic cellular response to the disease and the predilection for perivascular cellular concentration are found by all authors and are similar in mice, monkeys, and man.

In our studies of spleen changes, we found that early hyperplasia of the lymphoid-macrophage elements is accompanied, beginning on the fourth or fifth day, by a gradual reduction in the size of the Malpighian corpuscles and eventual cellular depletion. The generalized lymphoid-macrophage invasion of the connective tissue of the body begins on the fifth or sixth day, coinciding with (a) mobilization of cells in the spleen and probably lymph nodes, and (b) lymphocytosis in the blood stream. As the lymphoid organs become more and more depleted of lymphocytes and the excess is removed from the blood stream, the perivascular collections and organ-invading lymphocytes become more and more numerous and prominent.

PLATE 1

PHOTOMICROGRAPHS OF THE SPLEEN OF MICE EXPERIMENTALLY
INFECTED WITH *R. orientalis*, $\times 45$

FIG. 1: The normal control.

FIG. 2: Spleen one day post inoculation.

FIGS. 3 through 10 show the spleen on consecutive days from the third through the tenth day post inoculation.

Note that the general appearance of the spleens of the first four of these figures is quite similar. In figures 5 and 6, the follicles are slightly smaller in diameter and in figure 6, the outlines of the follicles are not as clearly demarcated. In figures 7, 8, 9, and 10, the general architecture of the spleen becomes increasingly distorted. In figures 9 and 10, no semblance of follicles can be made out and the lymphoid tissue is diffuse and exhausted.

PLATE 1

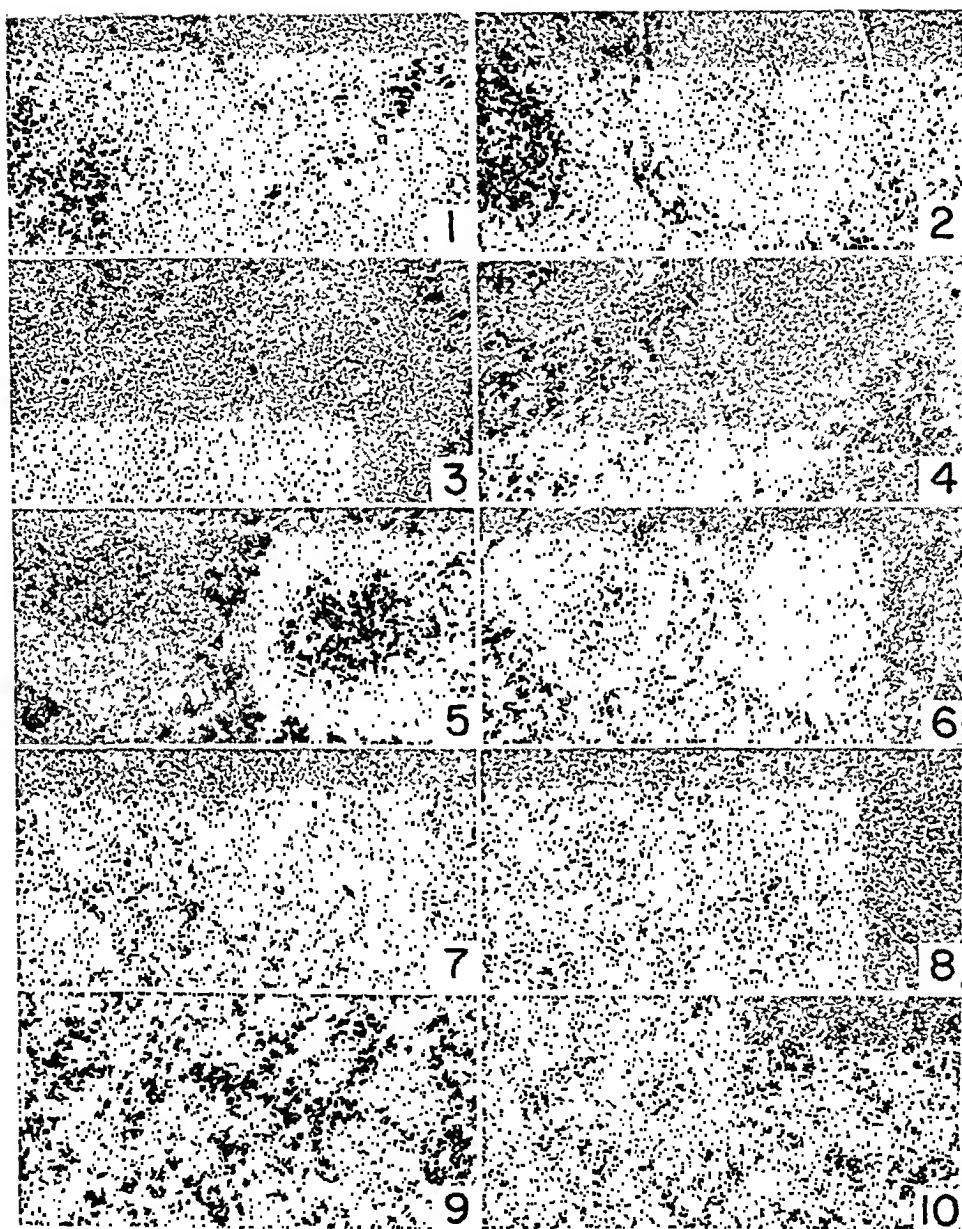


PLATE 2

PHOTOMICROGRAPHS OF HEART AND LUNGS OF MICE, MONKEYS, AND MAN
INFECTED WITH *R. orientalis*, X60

Heart (figure 11) of mouse shows a general infiltration of the connective tissue with lymphocytes. The cardiac muscle is normal.

Heart (figure 12) of sick monkey shows a pronounced focal collection of lymphocytes. There is no edema and the cardiac muscle appears normal.

Heart (figure 13) of man shows perivascular lymphocyte infiltration, slight edema and normal myocardium.

Heart (figure 14) of convalescent monkey contains only a mild diffuse lymphocytic infiltration in the connective tissue of the myocardium.

Lung (figure 15) of mouse is marked by a fairly prominent perivascular collection of lymphocytes and a slight thickening of the alveolar walls due to infiltration with lymphocytes and macrophages. Adherence of macrophages and lymphocytes to the endothelium of the blood vessels is prominent. The bronchi appear normal.

The lung (figure 16) of the sick monkey is very similar to the lung of the sick mouse described in figure 15.

Lung (figure 17) of man, in contrast to the sections of lung in experimental animals, shows an advanced interstitial pneumonia with marked thickening of the alveolar septa and frequent filling of the alveolae with edema fluid, red blood cells, and macrophages. The bronchi contain a few cells and edema fluid and their walls show moderate lymphocytic infiltration.

Lung (figure 18) of convalescent monkey brings out the thickening of the alveolar septa due to lymphocyte and macrophage infiltration but endothelial stickiness is absent, bronchi are normal, and the alveolar spaces are clear. Perivascular cell collections are fairly prominent.

PLATE 2

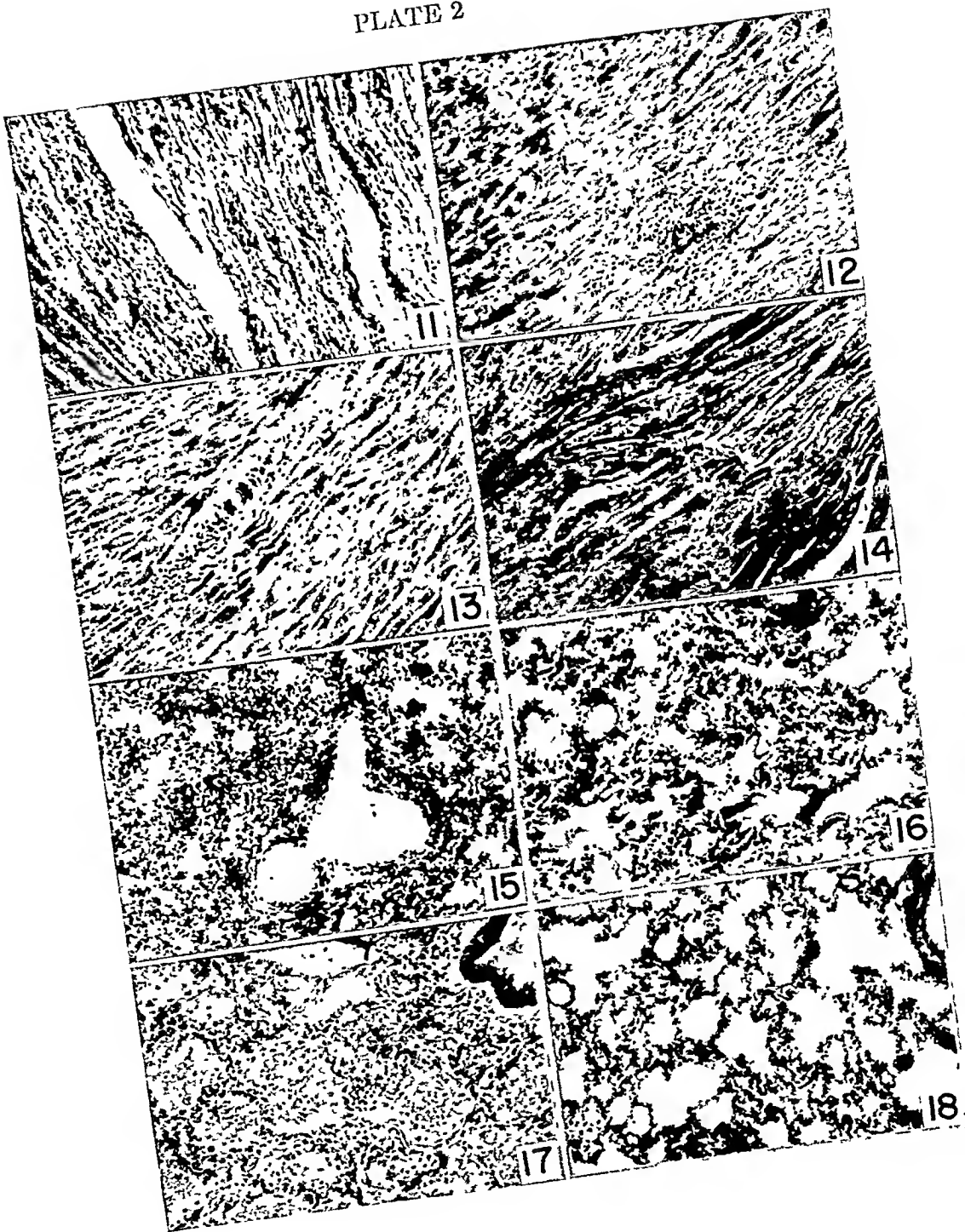


PLATE 3

PHOTOMICROGRAPHS OF LIVER AND KIDNEY OF MICE, MONKEYS, AND MAN
INFECTED WITH *R. orientalis*, X60

The liver (figure 19) of a sick mouse, shows adherence of lymphocytes to the endothelium of central and portal vessels and to the reticuloendothelium of the sinusoids. Perivascular collaring is sometimes marked. The sinusoids appear distended because of the perfusion technic. Liver cells appear normal.

The liver (figure 20) of a sick monkey is similar to the liver in figure 19.

The liver (figure 21) of man is marked by prominent periportal lymphocyte infiltration. The liver cells and sinusoids appear normal.

The liver (figure 22) of a convalescent monkey shows small focal collections of lymphocytes in the sinusoids and periportal regions and a few areas of liver-cell necrosis which, like the one illustrated, may become fairly large. Elsewhere, the liver cells appear normal. The areas of necrosis are believed due to large collections of round cells blocking the drainage in the sinusoids locally.

The kidney (figure 23) of a sick mouse shows marked diffuse and focal interstitial lymphocytic infiltration. Many of these collections are concentrated around blood vessels. Kidney parenchyma appears normal.

The kidney (figure 24) of a sick monkey shows interstitial and perivascular lymphocytic infiltration similar to that seen in the mouse.

The kidney (figure 25) of man is similar to those described in figures 23 and 24. In addition, casts are seen in some of the tubules. The tubular and glomerular epithelia appear normal.

The kidney (figure 26) of the convalescent monkey is similar to the other kidneys described. Tubules are free from casts. The infiltrating cells are chiefly lymphocytes and macrophages but in addition many plasma cells are seen.

PLATE 3

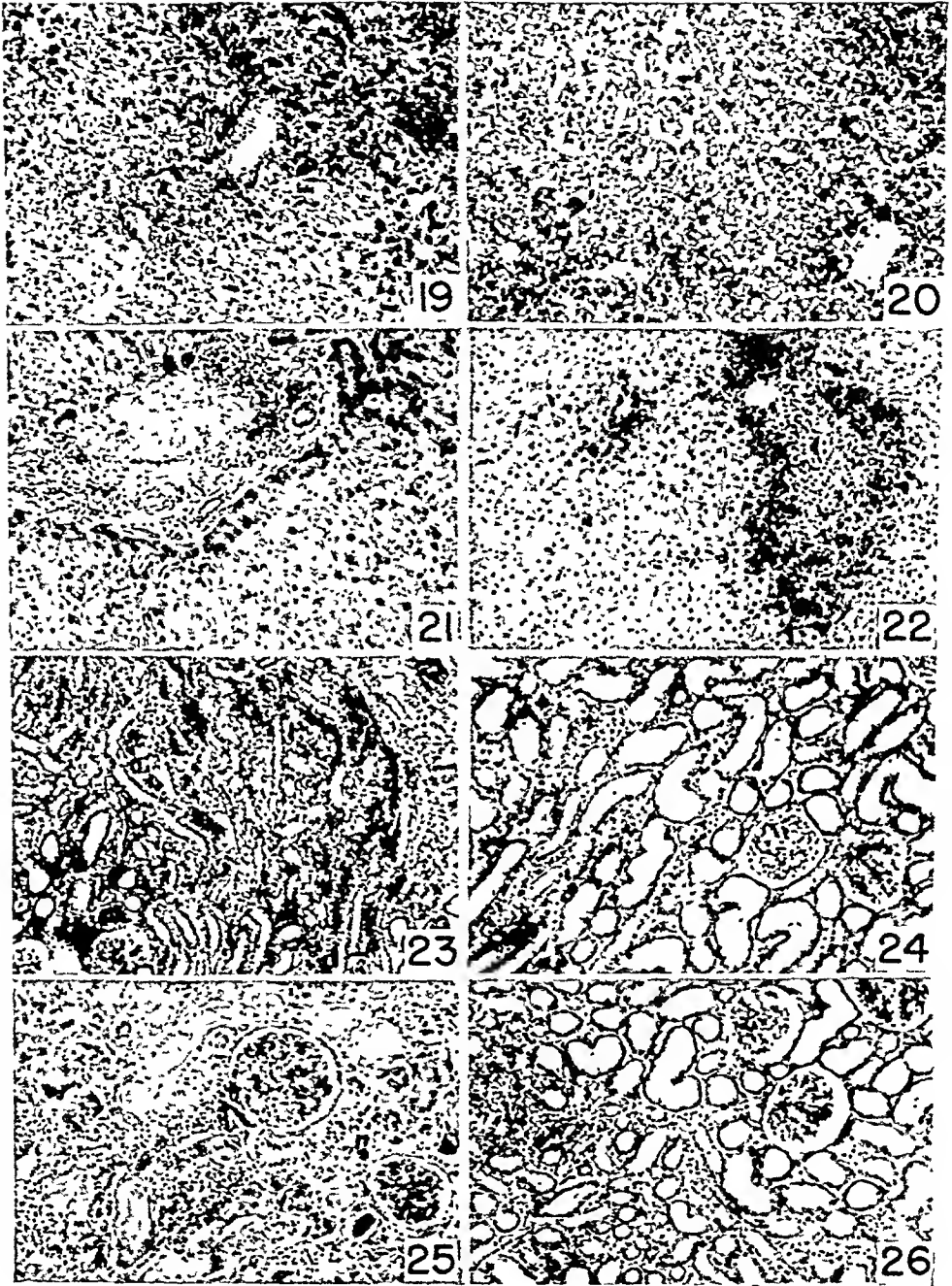


PLATE 4

PHOTOMICROGRAPHS OF THE BRAIN AND INTESTINE OF MICE, MONKEYS, AND MAN
INFECTED WITH *R. orientalis*, X60.

The brain (cerebrum) (figure 27) of a sick mouse shows several clumps of lymphocytes in the cortex and adherence of lymphocytes and macrophages to the endothelium of the vessels.

The brain (medulla) (figure 28) of a sick monkey shows a minimal reaction with a mild perivascular infiltration of lymphocytes in the pia-arachnoid. The brain substance appears normal.

The brain (pons) (figure 29) of man shows a prominent perivascular collection of lymphocytes and macrophages in the brain substance. Such areas are extremely rare.

The brain (brain stem) (figure 30) of a convalescent monkey contains rare foci of lymphocytes.

The large intestine (figure 31) of a sick mouse contains a very slight lymphocyte infiltration of the muscularis and in some areas of the submucosa. The epithelium and the lamina propria are normal.

The jejunum (figure 32) of a sick monkey shows a very slight lymphocyte and plasma cell infiltration of the lamina propria. In some areas not shown, perivascular lymphocyte collections in the muscularis are seen.

The large intestine (figure 33) of man shows a fairly pronounced lymphocyte, macrophage and plasma cell infiltration of the muscularis. This is not general but occurs in numerous small areas.

The large intestine (figure 34) of a convalescent monkey shows lymphocyte-macrophage infiltration of the muscularis and lymphocyte, macrophage and plasma cell infiltration of the lamina propria.

PLATE 4

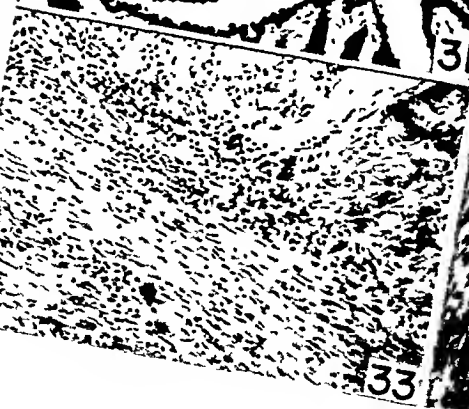
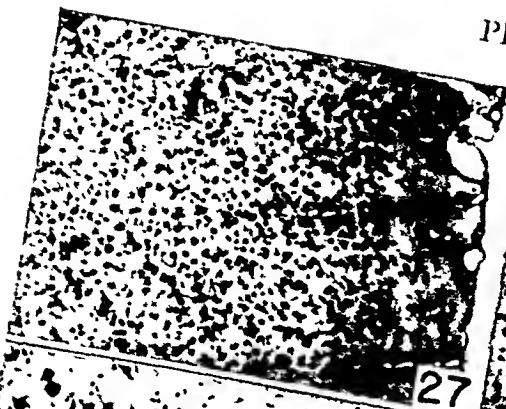


PLATE 5

PHOTOMICROGRAPHS OF SKIN ULCERS AND REGIONAL LYMPH NODES OF MONKEYS
AND PHOTOMICROGRAPHS OF MALPIGHIAN CORPUSCLES OF SPLEEN OF MICE
BOTH ANIMALS WERE EXPERIMENTALLY INFECTED WITH *R. orientalis*

The ulcer (figure 35), $\times 15$, formed at the site of inoculation in the skin of the monkey. This biopsy was taken on the eighth day post inoculation. The crust or scab that formed over the necrotic ulcer bed has been removed leaving the deep-seated ulcer which shows a tendency to undermining. The epithelium and a portion of the subcutaneous tissue are involved. Dilated blood vessels and intense polymorphonuclear infiltration are present in the subcutaneous tissue in the region of the ulcer.

The ulcer (figure 36) $\times 15$, removed from an inoculated monkey on the twelfth day post inoculation shows a similar picture except that healing has advanced. Subcutaneous tissue is being filled in with granulation tissue. The ulcer bed is rounded and shallow and the epithelium is growing over the margin. The regional lymph node (figures 37 and 38), $\times 280$ and $\times 60$, was excised from the monkey which developed the ulcer seen in figure 35. This lymph node is characterized by large numbers of macrophages which fill the lymph sinuses and which are very prominent in the nodules. Mitotic figures are common and the general impression is one of increased activity.

Portions of the Malpighian corpuscles of the spleens of infected mice are shown in figures 39, 40, 41 and 42, ($\times 580$). Figure 39 was taken the fourth day post inoculation and shows numerous mitotic figures and large lymphocytes. Figure 40 taken on the sixth day shows fewer mitotic figures, many large lymphocytes and many large reticular cells. In figure 41 taken on the eighth day post inoculation, large reticular cells outnumber the lymphocytes and mitotic figures are less frequent. On the tenth day post inoculation (figure 42), large reticular cells are prominent, lymphocytes are thinly spread, mitotic figures are about like those in figure 41.

PLATE 5

DIFF. 2

35

36

37

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42

PLATE 6

PHOTOMICROGRAPHS OF THE SPLEENS OF MICE, MONKEYS, AND MAN, AND OF THE
TESTICLE, KIDNEY PELVIS, PANCREAS AND LYMPH NODE OF MAN
INFECTED WITH *R. orientalis*, X60

The spleen (figure 43) of a sick mouse and the spleen (figure 45) of man in the terminal stage of disease are similar. The lymphoid tissue is diffusely dispersed and follicles are difficult to define.

In the sick monkey (figure 44) and the convalescent monkey (figure 46), normal architecture of the spleen is maintained. The monkey is able to combat tsutsugamushi fever and only rarely does one die after experimental inoculation. At comparable stages of the disease, the histological pattern of the spleen is entirely different from that in either mouse or man.

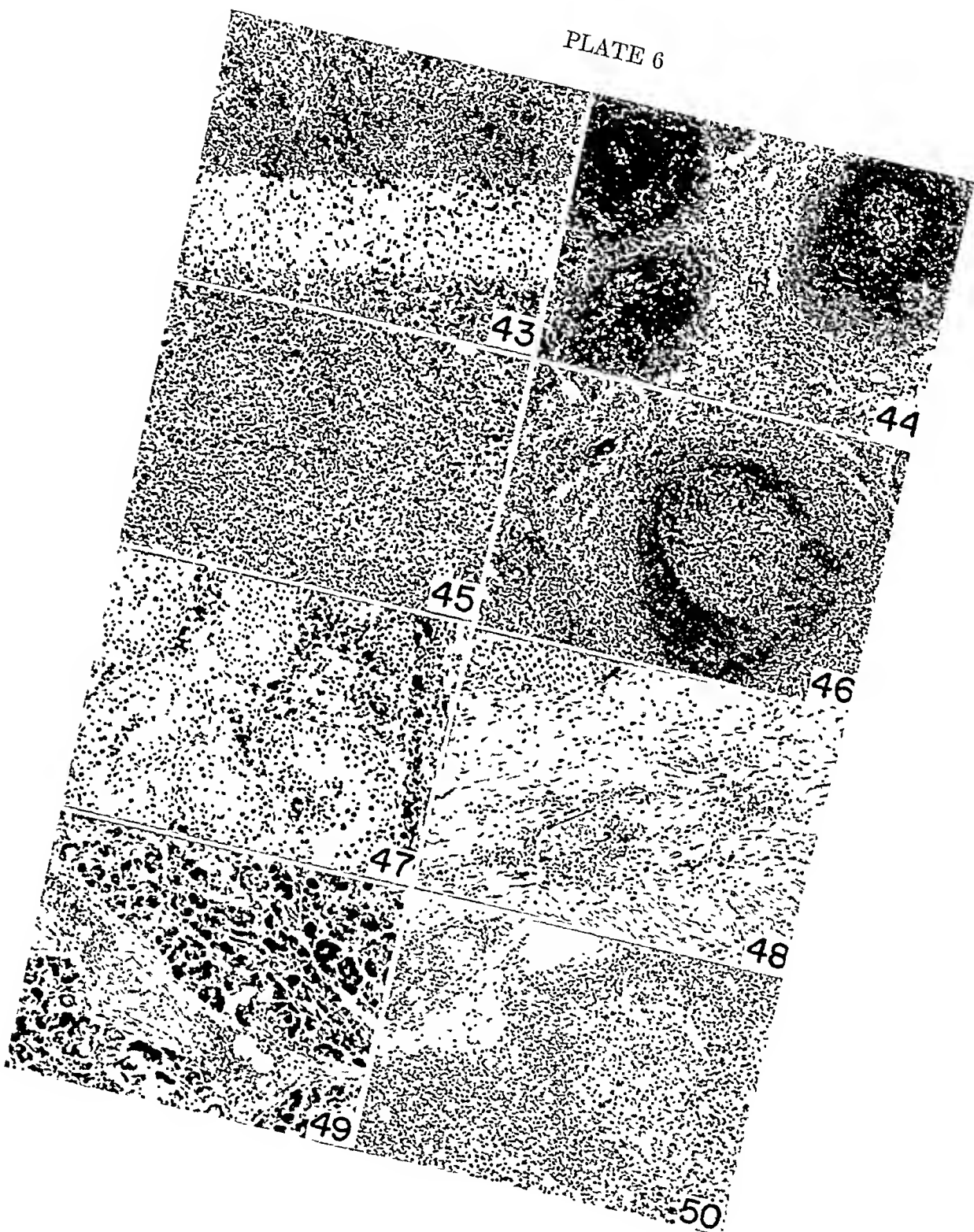
The testicle in figure 47 shows marked interstitial lymphocyte, macrophage, plasma cell infiltration. There is also very little evidence of spermatogenesis.

The kidney pelvis in figure 48 affords a fine example of perivascular lymphoid-macrophage collection plus some diffuse infiltration of the wall.

The pancreas (figure 49) shows a marked perivascular and mild generalized lymphocyte, macrophage and plasma cell infiltration.

The hilar lymph node (figure 50) shows perivascular lymphocyte and macrophage infiltration in a vessel of the capsule, a number of macrophages in the peripheral sinus and a nodule containing numerous macrophages many of which contain nuclear fragments.

PLATE 6



NEW CONCEPTS IN THE TREATMENT OF RELAPSING MALARIA¹

THE CHARLES FRANKLIN CRAIG LECTURE, 1946

JAMES J. SAPERO²

In this, the Eleventh Charles Franklin Craig Lecture, I have chosen to discuss a subject in malariology of urgent and practical importance, namely, the relation of treatment to relapse, in human malarial infections.

During World War II, the vast number of relapses and the confused attempts to find a curative treatment, clearly emphasized the great need for a better understanding of the relationships of relapse and treatment. Between one-half and one million individuals in the armed forces contracted malarial infections. The major proportion of cases (1) was caused by the South Pacific strain of *P. vivax*, notorious as one of the most persistently relapsing strains of any yet encountered. As an example, in one division of troops in the South Pacific there were 10,040 original admissions for malaria during an eight months' period of observation after withdrawal from Guadalcanal and during rehabilitation in a non-malarious area. Of these, 6,131 or 61 per cent relapsed one or more times. Approximately 27 per cent of the original group experienced two attacks, 9 per cent had three, and 2 per cent experienced four attacks. An occasional individual experienced a fifth and sixth attack during the eight months' period of observation. More extended observations have shown that subsequent to the eighth month, relapses in certain individuals continued into the second and rarely into the third year.

In attempting to effect radical cure of these cases, almost every conceivable form of chemotherapy was attempted. Quinine and atabrine were tried, first singly, and then in combination. Attempts to cure were next shifted to the combining of quinine, or atabrine, with plasmochin. Failing to reduce relapses by any of these methods, dosages were then increased, and prolonged frequently to the extent that patients were literally drenched with chemotherapeutic agents. The net result was that regardless of drug or combination of drugs, or the method of their employment, none of the various therapeutic modifications discernibly lowered the incidence of relapses.

The failure to prevent relapses during World War II was not unique. It was merely a reiteration of past experience. Throughout the history of malaria, it has been a characteristic of the disease that therapy, which so often is spectacularly successful in curing an individual attack of malaria, fails in the important objective of complete cure.

Still more confusing is the fact that treatment which succeeds in completely terminating infections in a large portion of one group, may utterly fail in another. One of the best illustrations of this is found in experiments conducted under the auspices of the Malaria Commission of the League of Nations (2). In these

¹ Presented at the Annual Meeting of the American Society of Tropical Medicine, November 6, 1946.

² Captain, Medical Corps, U. S. N. Malaria and Epidemic Disease Control Officer, South Pacific Areas and South Pacific Forces (1942-44), and of the Pacific Ocean Areas (1945-46).

studies, various observers in different countries, although using identical treatment regimens, reported remarkably discordant results. In one series relapse rates in the case of falciparum infections varied all the way from 16 to 90 per cent. In vivax infections, relapse rates varied from as small a figure as 21 per cent, to as high as 57 per cent. Further divergence was that in one area relapses of malignant tertian infections predominated over those caused by *P. vivax*; in another, the converse was true. Such wide discrepancies in relapse rates, even when similar treatment had been rendered, is illustrative of a situation which has been occurring and confusing clinicians for the past three centuries.

The crux of the situation, today, is that despite the very recent wartime development of new and more potent antimalarials, the problem of preventing relapses remains unsolved. This means that we must continue to deal with an age-old and exceedingly difficult situation. Irrespective of drug or method of treatment, a discouragingly large percentage of malarial infections can be expected to relapse, and to continue to relapse, often for months and years after the original attack.

The failure of wartime research to produce a *therapia sterilisans magna*, however, does not mean that therapeutic progress has not been achieved. Indeed, World War II has brought forth contributions of highest importance to the therapy of malaria. New facts have been revealed which tend to eliminate much of the confusion which has attended the problem of radical cure. Improper beliefs and false premises once generally taught are giving way to new and more rational concepts regarding the problem of relapses.

It now seems possible that a working hypothesis can be formulated which will largely explain the divergent and confusing results which meet attempts to obtain radical cure. Such an hypothesis promises an orderly pattern of thinking by which one may evaluate, and perhaps, even predict the therapeutic results likely to be obtained from any given regimen.

The discussion to follow will concern the formulation of such a working hypothesis. It will be seen that many of its elements are not new, being rather a reiteration of basic principles which unfortunately have been either lacking or poorly emphasized in texts on the subject of malaria. Other elements, however, are new, these being contributions of World War II research and practical experience.

BASIC FORMULA BY WHICH RADICAL CURE IS BROUGHT ABOUT

Although many an important hiatus exists in our knowledge it would seem that radical cure of malarial infections, in general may be expressed by the following formula:

Basic formula of cure in malarial infections			
Radical cure in an individual infection or Per cent of radical cures attained in a group	= Per cent efficacy of therapeutic regimen employed	+ Per cent efficacy of immune response of the individual or group	× Inherent characteristics of causative species or strain

This expression is not intended to be a precise, mathematical equation. It serves rather, to present a broad and general picture of the mechanism by which either cure or relapse may occur in malarial infections. As such, it has implications of highest clinical importance. The attainment of radical cure either in an individual infection, or the percentage of such cures in any group is not solely a matter of the exact therapeutic method employed as is so commonly believed.

Indeed, as the formula indicates, there are two other elements of major concern. These include a factor of immune response, and a factor in which the innate characteristics of the causative species and strain are concerned. It will be shown that the effect of either may suffice partially or completely to overshadow the effect of the single factor of specific therapy. As an example in point, it is well known that certain individuals who acquire malarial infections may experience spontaneous and complete cure even without the benefit of drug intervention. In such individuals, cure has been brought about solely by a potent immune response on the part of the host. The usual state of affairs, of course, is that a combination of both immune response and therapeutic intervention are essential for radical cure. Finally, there is the situation so commonly encountered, in which radical cure in certain infections is not attained despite the total curative effects of both therapy and immune response. In this group fall the cases of relapsing malaria. They represent individuals in whom the net result has led to relapse, for reasons such as deficiency in therapy, immune response or because of strain peculiarity. Since these three factors, alone or in combination, bear so significantly on the question of relapse, it is essential that each be examined in fairly intimate detail. This will be attempted in the discussion which follows.

FACTOR OF INHERENT SPECIES AND STRAIN CHARACTERISTICS

It is not stating a new principle to say that the four species of plasmodia which cause human malaria have distinctive and inherent qualities (2, 3, 4). The matter of concern, judging by World War II experience, is that very few physicians seem to appreciate the nature of these specific differences. An attempt will be made to define the natural course which infections tend to pursue as a result of innate qualities, and to show how these natural tendencies strive to assert themselves irrespective of treatment or other influence.

Present available evidence suggests that the principle involved may be stated as follows: *Individual species of plasmodia and certain individual strains of a single species show inherent differences: (a) in their pattern of relapse in the human host; and (b) in their susceptibility to drugs.* Both of these qualities tend to assert themselves more or less characteristically, despite such opposing influences as host immunity or drug therapy.

An outstanding example of inherent differences which exist among the various species of plasmodia is afforded by *P. ovale*. This species is distinctive in that infections caused by it show little tendency to relapse, and exhibit a high degree of drug susceptibility (4). This latter is indicated by the fact that remarkably small doses of quinine usually suffice to bring about radical cure of the infection.

P. ovale infections also serve excellently to demonstrate how the inherent

peculiarities of a particular species of malaria may dominate over all of the other factors concerned in the formula of cure. Thus, 100 per cent radical cures are generally obtained even with therapy which ordinarily would be considered subminimal for other species of malaria. Further there is the fact that the remarkable curative results are largely attained irrespective of the variable immune response of infected individuals.

P. vivax infections, in contrast to those caused by *P. ovale*, exhibit a marked tendency to relapse. Commonly overlooked by many clinicians is that there is a particular pattern of relapse (4) which the vast majority of strains of *P. vivax* appear to follow with considerable uniformity. In this regard the timing of relapse and the span of infection are both of importance. The usual course in vivax infections is that very shortly after recovery from the primary attack, a series of relapses takes place. Then there follows a period of clinical latency. Usually this lasts from seven to eleven months, after which another series of relapses may occur. The life span of vivax infections usually extends for a period of a year or more.

It has become well established that not only do the species exhibit distinctive qualities but that such are also observed in the case of certain individual strains (2) of *P. vivax*. In the matter of drug susceptibility the recent experiments of Earle (5) emphasize that strains may vary considerably in their response to therapy. In McCoy vivax malaria complete eradication of trophozoites with paludrine was achieved by a minimum plasma drug level of 10 micrograms per liter maintained for four days. Using the same drug with the Chesson strain of vivax malaria, a plasma level of greater than 20 micrograms per liter, or twice that required with the McCoy strain, was found necessary.

Remarkable evidence of the distinctive qualities between strains of a single species is exhibited by the St. Elizabeth and the Chesson strains of *P. vivax*. The mosquito inoculation into human subjects with the St. Elizabeth strain (a strain indigenous to the United States) has revealed a pattern of reactivation and drug susceptibility which might well serve as a basic type with which all other strains of *P. vivax* can be compared profitably. Using the St. Elizabeth strain, Coatney, Cooper and their collaborators (6) at the National Institute of Health have shown that the primary attack is uniformly and readily susceptible to cure. Recrudescences do not occur after the primary attack, but with great regularity there ensues a clinically latent period of seven to eleven months. Renewed clinical activity in the form of a series of one or more relapses thereafter takes place almost without exception. Finally, in most individuals the life span of infections with the St. Elizabeth strain has not exceeded fourteen months' duration.

The response of the St. Elizabeth strain in the human host is similar to the pattern exhibited by the majority of all studied vivax strains. Unlike the St. Elizabeth strain, however, many other strains of the species (4, 5) show a series of recrudescences shortly after the primary attack and a much lesser tendency to late relapses. It is possible that this variation from the basic pattern of the St. Elizabeth strain is indicative of innate relapsing tendencies in at least certain

of these strains. Without further study, however, one cannot say that the variable patterns are not in reality due to the influence of host immunity or to therapy.

In contrast to the pattern which the St. Elizabeth strain presents, is the very distinctive pattern of the Southwest Pacific strain of *P. vivax*. Practical experience in the field reveals a pattern of relapse which may extend over a period of two or more years. Human experimentation (6) with the Chesson strain (Southwest Pacific) does not show such longevity. Under both experimental and natural conditions, however, reactivation occurs throughout the period of the infection with neither the discernible tendency to early and late relapses, nor the intervening seven to eleven months' period of latency, so characteristic of the St. Elizabeth strain. The Southwest Pacific strain exhibits this distinctive pattern under conditions of study identical to those employed with the St. Elizabeth strain. This leaves little doubt that the two strains are inherently different.

In marked contrast to vivax malaria, the most important aspect of falciparum infections is that these seldom persist for more than six months. The numerous falciparum strains carefully studied by James *et al.* (7) in England, and by Boyd, all follow a pattern of being relatively short-lived. As Boyd has shown (8) in summarizing these studies, renewed clinical activity after the tenth week of infection is relatively uncommon. Recrudescences, following shortly after the primary attack occur commonly. Relapses, so characteristically seen after long periods of latency in vivax infections, are not a part of the picture in falciparum malaria. However, there are marked differences between strains in regard to the number of relapses which occur. Boyd (8), for instance, reports 8.3 per cent relapses, while 80.6 per cent relapses occurred in the strains studied by James (7). This variability appears largely ascribable to inherent differences in regard to drug susceptibility. Numerous examples of this have been reported in the literature. The most noteworthy is by James (7) in which two of his falciparum strains, in comparison with 34 others, required 8 times the amount of quinine to bring about satisfactory clinical control.

It is important to note that in addition, strains of falciparum show a much greater drug resistance than strains of *P. vivax*. This has been confirmed both by clinical experience and experimental studies (2, 4, 5, 9). A very recent example in point is that Earle (5) testing paludrine against the Costa strain of *P. falciparum* has found that it requires in excess of 100 milligrams of paludrine per liter to eradicate trophozoites. This is of course greatly in excess of that required in experiments for trophozoite eradication in either the McCoy or Chesson strains of *P. vivax*.

Regarding the fourth species of human malaria, *i.e.* *P. malariae*, so little is known of its relapse pattern and drug susceptibility that it does not seem profitable at this time to attempt a discussion of its inherent characteristics.

It is of interest that the characteristics of species and strains which have just been discussed appear to be of a remarkably stable nature. As an example, repeated passages through a series of either anopheline or human hosts, do not bring

about discernible changes in the inherent relapse pattern of a given strain (3); similarly, the quality of drug susceptibility does not seem to change even after prolonged administration of any given antimalarial drug.

Returning now to the basic formula of cure, it is evident that quite apart from the factor of therapy or of immune response, the innate qualities of the specific infective organism play an exceedingly important role in the final determination of cure. Failure to cure may follow because of the particular qualities which the strain itself exhibits rather than the lack of efficacy of a given scheme of therapy. With this in mind we gain an insight into the cause of much confusion in the past. It becomes obvious that methods of treatment which have been advocated enthusiastically in one place, in another locality may fail miserably. In the past the clinician has tended to question the treatment, and then to revise or to invent a new therapeutic scheme. The trouble, of course, may lie almost entirely in a biological character of the individual species or strain.

The importance of the role of inherent species and strain characteristics makes it almost mandatory that clinicians become familiar with the biological nature of the various strains of malaria which they may be called upon to treat. Assistance of malariologists, of course, must be forthcoming in the matter of delineating clearly the number and nature of indigenous strains wherever the disease exists. This is a study in which unfortunately only the very surface has been scratched.

PROBABLE EXPLANATION FOR THE MECHANISM OF SPECIES AND STRAIN DIFFERENCES

Before proceeding to a consideration of the two other major factors of the formula, namely, therapeutic efficacy and immune response it seems timely to inquire into the probable mechanism by which distinctive relapse patterns are exhibited by various species and strains of plasmodia. A consideration of this introduces a hypothetical principle of great importance to the over-all concept.

This principle postulates the existence of an exo-erythrocytic cycle in man, and, therefore, that *relapses in malarial infections are of two types: those which follow as a result of parasitic activity in the exo-erythrocytic cycle and those which follow from renewed parasitic activity in the conventional erythrocytic cycle.*

In 1902 Schaudin claimed to have observed the direct invasion of red cells by sporozoites. Upon his authority and without confirmation, direct sporozoite invasion of erythrocytes was accepted as a factual occurrence. Thereafter, almost total emphasis in teaching has been placed on the periodic cyclic development of parasites in red cells of the blood stream. As a corollary to this concept, it has been believed generally that relapses occur solely from parasitic activity in the erythrocytic cycle.

Recently, the consensus of most malariologists seems to be that this simple concept of relapse must be abandoned. The term, "exo-erythrocytic relapse," now holds an important place in a new and broader concept. In this concept relapses may be either "erythrocytic" or "exo-erythrocytic" in origin. The mechanism of the former will be considered in a subsequent discussion; that of the latter, *i.e.*, exo-erythrocytic relapse, will be dealt with at once.

The newer version of parasitic activity in man is that following inoculation, sporozoites enter fixed-tissue cells. Within fixed-tissue cells, it is thought probable that the parasites undergo a distinct cycle of development, a so-called exo-erythrocytic cycle. Subsequently, parasites are discharged into the blood stream and there begin the erythrocytic cycle.

Further, it is now postulated that persistence of infection, and relapse, are both closely linked with the exo-erythrocytic development of parasites. Relapses are conceived to follow periodic discharges of tissue forms into the blood stream. The persistence of infection, on the other hand, is held to be an expression of the total period during which the periodic expulsion of fixed-tissue forms may continue.

Such a concept offers a logical explanation of the mechanism by which both species and strains differ inherently as regards relapse pattern and the length of infection. It would appear, for example, that in falciparum malaria the infection is generally short-lived, due to complete expulsion of all the parasites from the fixed tissues within a period of a few weeks. In vivax infections, in contrast, a prolonged period of continued activity in fixed tissue occurs. Repeated periodic expulsion of parasites into the blood stream continues for months and years, terminating only when all the parasites in the underlying tissues are expelled.

Regarding the variable relapse patterns seen in strains of a single species, the concept accounts for these as distinct and innate expressions of parasitic activity originating in the exo-erythrocytic phase.

It is true that conclusive proof of the existence of an exo-erythrocytic cycle in man has not been demonstrated. The finding of exo-erythrocytic forms in several of the avian malarias, has strongly suggested the possibility of their existence in man. To date, a few observers have claimed to have seen such forms in the human host. Obviously, however, extensive and indisputable confirmation will be required.

There is much to support the hypothesis in the way of indirect evidence. Boyd (8) has reported that his own experiments and those of others have shown that after mosquito inoculation of sporozoites, sub-inoculations of blood fail to produce malaria in the recipient earlier than the fifth day. This finding almost surely rules out the possibility that direct, and immediate invasion of red cells occurs. On the contrary, the indication is that sporozoite invasion of fixed tissue constitutes the initial event which takes place.

Recently Fairley (10) has shown that blood transfusions, performed within seven minutes after bitings by anophelines infected with either *P. vivax* or *P. falciparum*, resulted in the transfer of infections to the recipients. This indicates that sporozoites actually do enter the blood stream. However, it is only a matter of minutes until they leave, for after thirty minutes, transfusions then fail to produce infection. This situation in which parasites are absent from the blood stream, continues for exactly six days in falciparum infections, and for precisely eight days in vivax infections. Thereafter, transfusions uniformly cause malaria in the recipients. This indicates that reinvasion of the blood stream finally has taken place.

Fairley believes that the sharp demarcation between negative and positive

results obtained upon transfusion, namely, the invasion of the blood stream exactly on the seventh and ninth day by *P. vivax* and *P. falciparum*, respectively, has important connotations. He suggests that four cycles may occur in the fixed tissues in vivax infections, and three in falciparum, before the parasites enter the circulation.

Recently another supporting observation is that reported by Coatney and Cooper (6). They were interested in determining whether parasites were lying dormant and at undetectable densities in the blood stream during periods of latency. In ten patients infected with the St. Elizabeth strain by mosquito inoculation, sub-inoculations of from 250 to 300 cc. of blood into susceptible individuals were performed during the mid-point of the usual seven to eleven months' period of latency. Nine of the recipients remained negative. This indicated that dormant parasites were not in the blood stream. All nine donors experienced late relapses after the usual seven to eleven months' period, proving that all had been harboring latent infections at the time of sub-inoculation. This being so, the experiment strongly suggests that these late relapses were expressions of exo-erythrocytic activity.

The tenth case, interestingly enough, did not negate the probable explanation of events in the other nine cases. In this case, unlike the others, both donor and recipient experienced clinical attacks of malaria at about the same time. It would thus appear that in the donor an expulsion of the underlying tissue forms into the blood stream had occurred by coincidence at the time of sub-inoculation.

Whatever objection one might have to the lack of conclusive proof regarding the existence of an exo-erythrocytic cycle in man, the fact remains that tentative acceptance of the version brings to an understandable basis a whole series of events which previously held concepts left unexplained. It provides a plausible explanation of the mechanism by which different distinctive patterns of relapse occur in malarial infections. In the section to follow we shall see another connotation of even greater importance. There is distinct promise that completely curative treatment may be achieved by therapy aimed at the prevention of exo-erythrocytic relapses, as well as relapses of erythrocytic origin.

FACTOR OF CHEMOTHERAPEUTIC EFFICACY

Important in the consideration of the various elements which determine chemotherapeutic efficacy is an understanding of the selective activity of drugs, and inherent differences which exist in their plasmocidal capacity. These factors assume particular importance in their different relationships to exo-erythrocytic and erythrocytic types of relapse.

A principle of importance is as follows: *Chemotherapeutic agents in general use in malaria exhibit a highly selective action. Drugs capable of eradicating trophozoites of the erythrocytic cycle are incapable of a like action on pre-erythrocytic forms. Conversely, drugs potent against pre-erythrocytic parasites are largely without effect on erythrocytic forms.*

In general, it has been found that quinine, quinacrine and chloroquine, so far as practical benefit against asexual forms is concerned, are active only upon

erythrocytic forms. In contrast, plasmochin, and a new and closely related drug, pentaquine, (11) act exclusively upon exo-erythrocytic forms.

This selective activity is supported by various observations (12, 13). In the treatment of blood-induced malaria in which, as we shall see presently, it is almost certain that only erythrocytic activity occurs, response is poor or absent if treatment is attempted with drugs of an exo-erythrocytic activity. On the other hand, in naturally acquired infections, drugs active against erythrocytic forms have no therapeutic effect if administered during the incubation period while the parasites are solely in the fixed tissues. In contrast, drugs with exo-erythrocytic activity when administered before parasitic invasion of the blood stream has occurred have effected radical cure.

This selective activity has implications of considerable importance. In the past the major efforts in attempting curative treatment have been by the use of drugs capable only of preventing erythrocytic relapses. The newer approach demands as well that therapy be directed towards the prevention of the exo-erythrocytic type of relapse. Either a single drug must be discovered which has the dual capacity of acting both against the parasites of exo-erythrocytic and erythrocytic phases, or a combination of two drugs which will accomplish the same result must be used.

It is of interest that paludrine (14), a synthetic antimalarial of British origin, appears unique since it represents the first discovery of a drug which has appreciable dual activity against both erythrocytic and exo-erythrocytic forms. However, investigations (15) to date indicate that exo-erythrocytic forms are the more susceptible. The effects on erythrocytic forms are less promising. Comparative tests will be needed to determine whether paludrine in practice will serve as an efficient drug against both forms. So far it appears that against strains of falciparum infections, tested by Fairley (15), it can eradicate the exo-erythrocytic forms and thus effect radical cure. The effect against the Southwest Pacific vivax strain is only partial in that radical cures are by no means regularly attained.

Some success also has been achieved by the simultaneous use of two drugs. Thus, recently, trials (11, 16) have been made by combining quinine with plasmochin or pentaquine. This combination provides an attack on forms of both cycles. It has led to complete cure in vivax infections in an appreciable number of instances. The trials confirm previous claims (4) that plasmochin combined with quinine, will reduce the number of relapses. The difficulty in the past (16) has been that too often the combined treatment failed. It seems probable now that failures were due to using inadequate doses of plasmochin. The required dosage of plasmochin is near or at a seriously toxic level. Smaller, safe doses are apt to be partially or wholly ineffective. Pentaquine appears less toxic, but like plasmochin is possibly too dangerous to permit this otherwise ideal form of combined treatment to be brought into general usage.

A point of practical importance is that in falciparum infections radical cure may be effected by using a drug solely capable of destroying erythrocytic forms (10, 16). The mechanism is one in which effective blood levels are maintained

throughout the entire period in which it is presumed that the underlying tissue forms are being expelled into the blood stream. The fixed-tissue forms are not affected, but upon expulsion into the blood stream as erythrocytic forms, are eradicated by a drug which is active against these forms. The same practical end-result is attained as in radical cure.

It is feasible to accomplish radical cure when the span of exo-erythrocytic activity is short as in falciparum infections. Indeed it seems likely that the administration of drugs active against erythrocytic forms, throughout the entire span of vivax infections, if such prolonged treatment were feasible, also would effect the same end-result of complete cure. In this connection Coatney and Cooper (6) have shown with the St. Elizabeth strain that by proper timing and anticipation, a drug capable of acting against erythrocytic forms may be employed to eliminate individual relapses.

The new approach concerned with the necessity of terminating the exo-erythrocytic type of relapse still leaves the problem of erythrocytic relapse. That these latter occur and are of a type distinct from those of exo-erythrocytic origin is strongly suggested by the very different behavior of blood-induced and sporozoite-induced infections. In the former, relapses tend to be less in number, late relapses are absent, and usually, with only small amounts of drug, complete termination of the infection is possible. These are quite different characteristics from those seen in sporozoite-induced or naturally-acquired infections. The suggestion is that the erythrocytic type of relapse follows from a separate and independent mechanism of parasitic activity.

As Shannon and his co-workers (17) have shown in a series of brilliant pharmacological investigations, the therapeutic effectiveness of drugs active in the erythrocytic cycle varies according to inherent plasmocidal capacity. Of concern is the degree and promptness with which effective blood plasma levels are reached and the length of time these levels are maintained. Further is the fact that no two individuals are apt to attain identical levels even though identical doses have been taken. Facts such as these must now be learned in detail if erythrocytic types of relapse are to be minimized.

A final matter for consideration is the need for distinguishing between erythrocytic and exo-erythrocytic reactivation. We can do so only in a very broad sense. In a given infection where renewed clinical activity occurs after a few days of latency (recrudescence), such activity represents, in all probability, the erythrocytic type of reactivation. On the other hand, reactivation (relapse) occurring after a latent period of several months is almost surely of exo-erythrocytic origin. The nature of episodes which occur between these two extremes is not known at present. Obviously, there is an urgent practical need for experimental studies which will clearly delineate between recrudescences and relapses, whatever the time of their occurrence.

THE FACTOR OF HOST IMMUNITY

Most of the emphasis regarding immunity in malarial infections is that the immune mechanism operates too slowly and too ineffectively to be relied upon by the clinician seeking radical cure. What is apt to be overlooked, is that while

the physician himself cannot often stimulate or regulate immune response, defense reactions nevertheless take place and vitally affect the relapsing tendency of the disease.

Many examples may be cited to prove the potency of the immune factor in its effect upon relapse. As an example, if a drug or method of treatment were tested in the southern United States against *P. vivax* infections, the resultant relapse rates very much more likely would measure the immune response of the group rather than the efficaciousness of treatment. This follows from observations (18) that the southern negro is relatively refractory to vivax infections and that relapses are less apt to occur with the frequency encountered in whites. Again, if the test is conducted in a group of individuals who had previously suffered from malaria, the resultant tolerance (8) which develops under such circumstances could be expected to modify the relapse rate very materially. Then always there exists to a variable degree, such factors as intercurrent disease, sudden climatic changes, alcoholism, and other stimuli which are presumed capable of precipitating relapses. The operation of factors such as these, once more demonstrate that the exclusive focus of attention on therapy rather than upon all of the factors in the formula of cure, may lead to unjustified conclusions.

There is still another factor which recently has attracted much attention and which emphasizes the predominant role immunity may assume in regard to relapses. This is the observation (19) that both the length of the prepatent period and the tendency to relapse in malarial infections varies with the dosage of sporozoites. With a small inoculum the prepatent period is prolonged and the liability to relapse greatly diminished; with a larger inoculum the converse is true.

So far as lesser tendency to relapse is concerned, this suggests that important immune activity occurs very early in malarial infections, namely, while the sporozoites and next succeeding forms are in the tissue phase. The phenomenon appears similar to that so frequently seen in other infections. Thus a small inoculum usually stimulates the more potent defense, while a large inoculum tends to reduce or may even overwhelm the defensive mechanism.

SUMMARY AND CONCLUDING REMARKS

It has been pointed out that despite the magnificent contributions of wartime research towards the producing of new and more efficacious antimalarial drugs, the ideal drug capable of radical cure remains to be discovered. This means that the practicing physician must continue to deal with antimalarial agents known to be of sub-optimal efficacy—that ways and means must be devised of attaining the best possible effects from drugs only partially effective in overcoming the notorious relapsing tendency of malarial infections.

Attempts to discover the most efficacious scheme of treatment however have in the past led to much confusion and controversy. This is to be expected for frequently, identical therapeutic schemes, when repeated on different groups, have produced amazingly different results.

The objective in this lecture, then, has been to examine the probable under-

lying causes which have led to discrepancies and confusion in therapeutic evaluation.

To emphasize the major factors which seem to be concerned, a formula of radical cure has been devised. The formula states that the probability of radical cure in an individual, or of cures in a group is determined by the effectiveness attained by the factor of drug efficacy plus that attained by the factor of host immunity. The net effect of these in turn is reduced or augmented by a third factor, the inherent characteristics of the species or strains of malaria concerned.

The formula is not intended as a precise statistical method which will determine the likelihood of cure. Rather it purports to express the broad and general principles which are concerned in the mechanism of cure. Its workings for illustrative purposes, however may be demonstrated arithmetically. For example if inadequate treatment, roughly of 25 per cent efficaciousness is given, and if immune response similarly is about 25 per cent, the net result of these two factors alone would give about 50 per cent cures. This percentage, however, when multiplied by the third factor, might be either doubled or halved. Thus 100 per cent cures might still follow if the strain concerned were drug susceptible, or there might be only 25 per cent cures, if the strain were inherently drug resistant. This suggests how variably the mechanism of cure may operate to produce almost any given curative result.

The formula emphasizes: (a) That results achieved by any given system of treatment may be dominated by factors quite independent of the treatment itself; (b) if identical regimens of treatment are employed in different groups, variable results are inevitable, for only by coincidence could either of the other two factors operate in an identical manner; (c) that as a result, divergent results which follow in trials evaluating treatment, actually should not be confusing, but are to be expected; and finally, (d) that the common recourse which is so often taken when a plan of treatment fails, such as doubling or prolonging dosage or otherwise revising standard forms of treatment, should be undertaken only when one is convinced that the true explanation of failure does not rest in the operation of other equally influential factors.

Finally, a full consideration of the problem of preventing reactivation seems to demand not only the broader concepts which the formula suggest, but in addition, an understanding of many newer concepts by which each of the various factors in the formula tend to express themselves. Among the most important of these is the postulate that an exo-erythrocytic cycle exists in man; and the corollary, that two types of reactivation occur: exo-erythrocytic (relapses) as well as erythrocytic (recrudescences). In regard to therapy which aims at radical cure, the use of drugs capable of both exo-erythrocytic and erythrocytic activity appears to be essential. In regard to immunity sporozoite dosage looms as of new interest and of great importance is the pattern of clinical activity in the human host. Drug susceptibility and the pattern of relapse appear to be the essential elements peculiar to species or strains of species which are important in determining whether cure or clinical reactivity will follow.

These are matters, then, that constitute new concepts which, in turn, promise

to provide a more rational and better understanding of the many difficult problems involved in the treatment of relapsing malaria.

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POLYVALENT ANTIGENS FOR THE DIAGNOSIS OF SALMONELLOSIS IN DIFFERENT CLIMATES¹

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About 130 *Salmonella* strains are described to date. Any of these strains may cause human salmonellosis. This disease presents itself as *Salmonella* fever (also called typhoid-like fever), septicemia, enteritis or a localized affection. While the isolation of salmonellae from the patient does not, as a rule, cause difficulty, in a certain number of cases serologic tests are necessary to establish the diagnosis of the disease. Such diagnostic procedures, however, are greatly impeded by the multitude of the "O" and "H" antigens of salmonellae. 38 "O" and 70 "H" antigens have been accepted to date. It is impossible to test for all antibodies corresponding to these antigens in the routine laboratory. Attempts were made, therefore, to set up diagnostic procedures whereby the serum of the patient is being tested against only the most important antigens.

A commonly accepted procedure is to attempt agglutination with typhoid, paratyphoid A and paratyphoid B antigens. This method gives positive results in but few of the cases of salmonellosis encountered in the United States, because *Salmonella* strains frequently encountered, as *S. montevideo*, *S. oranienburg*, *S. cholerae-suis* etc. do not have common antigens with the above enumerated organisms, while, on the other hand, paratyphoid A infections are very rare in North America.

Bornstein (1942) therefore recommended the use of a limited number of antigens, covering the most important *Salmonella* organisms encountered in the United States.

When such polyvalent *Salmonella* antigens are prepared, the geographic distribution of salmonellae has to be kept in mind. Table 1 shows the most frequent *Salmonella* strains encountered in different parts of the world. It was compiled from the data of Edwards and Bruner (1943), Bornstein (1943), Seligmann *et al.* (1943), Felsenfeld (1945), Rubinstein *et al.* (1944), Hajna and Perry (1945), Galton and Quan (1943, 1944), Morris *et al.* (1944), Godelfer (1945), Kessel *et al.* (1945), Fulton (1945), Neter (1944), Neter and Clark (1944), Felsenfeld and Young (1944) and Young (1944) on *Salmonella* types in the United States; Hormaeche *et al.* (1943), Stone (1943), Varela and Zozaya (1944) and Vaccaro *et al.* (1943) in Central and South America; Kauffmann (1941), Savage (1942), Naarhoff (1941), Hohn and Herrmann (1940), and Maccolini (1941) in Europe; Bruner (1945), Cossery (1946) and Olitzki (1945) in the Mediterranean area; Henning *et al.* (1942) and Lewin and Roux (1945) in Africa; Karunakaran and Pillai (1942), Hayes and Freeman (1945) and Kauffmann (1941) in India and Draper (1945), Atkinson (1946), Fortune and Ferris

¹ Read at the Annual Meeting of the American Society of Tropical Medicine. Miami, November 4-7, 1946.

(1945), Jacoby *et al.* (1945) and Lindberg and Bayliss (1946) in Australia and on the Pacific Islands. The most frequently encountered strains are listed in the table according to their position in the Kauffmann-White schema of salmonellae. In table 1, twelve frequent *Salmonella* strains are enumerated for each area of the world. The polyvalent antigens most efficient for use in any country have to include agglutinogens representing all salmonellae occurring in this table.

Table 1 shows that the task of constructing polyvalent *Salmonella* antigens

TABLE 1
Salmonella types most frequently encountered in different parts of the world

GROUP	SALMONELLA	ANTIGENIC STRUCTURE	IN AMERICA	IN EUROPE- AFRICA	IN ASIA- AUST- RALIA
A	<i>paratyphi A</i>	(I), II, XII: a—	I	F	F
B	<i>paratyphi B</i>	(I), IV, (V), XII: b—1,2	F	F	F
	<i>typhi-murium</i>	(I), IV, (V), XII: i—1,2,3	F	F	F
	<i>derby</i>	(I), IV, XII: f,g,z ₈	F	I	F
C ₁	<i>paratyphi C</i>	VI, VII, (Vi): c—1,5	I	I	F
	<i>cholerae-suis</i>	VI, VII: (c)—1,5	F	F	I
	<i>thompson</i>	VI, VII: (k)—1,5	I	F	I
	<i>oranienburg</i>	VI, VII: m,t—	F	F	F
	<i>bareilly</i>	VI, VII: y—1,3,4,5	F	I	I
	<i>montevideo</i>	VI, VII: g,m,s,z ₁₂ z ₂	F	I	F
C ₂	<i>newport</i>	VI, VIII: (e,h)—1,2	F	F	F
	<i>morbificans bovis</i>	VI, VII: r—1,5	I	F	I
D	<i>typhosa</i>	IX, XII, (Vi): d,d ₁	F	F	F
	<i>enteritidis</i>	(I), IX, XII: g,o,m,z ₁₂ z ₂	F	F	F
	<i>dublin</i>	I, IX, XII: g,q	I	F	F
	<i>panama</i>	(I), IX, XII: l,v—1,3,4,5	F	I	I
E ₁	<i>london</i>	III, X, XXVI: l,v—1,4,6	I	F	I
	<i>anatum</i>	III, X, XXVI: e,h—1,4,6	F	I	F

Remarks: F = frequent

I = infrequent or as yet unrecorded.

can be enhanced when "O" and "H" antigens are considered separately. There is no great necessity to use group "A" antigen (Roman numerals in the antigenic formula of *S. paratyphi A*) in America. It is the experience of the author, confirmed by Karunakaran and Pillai (1924), that in paratyphoid A infections the agglutination reaction with the "O" antigen is often very weak. One has to rely on the flagellar ("H") agglutinin in the serodiagnosis of paratyphoid A. It is necessary, however, to use the "O" antigens of the other frequently occurring groups, even if we do not expect positive results in the beginning of infections with organisms carrying a Vi antigen. The polyvalent "O" agglu-

tinogen, therefore, will be composed of the group- antigens B, C₁, C₂, D and E₁.

The "H" antigen is composed of a specific (designated with Latin letters) and a non-specific (designated with Arabic numbers) phase. As to the non-specific phase, the groups of 1, 2; 1, 5 and 1, 4, 6 are the most frequent and therefore have to be used in serologic tests.

Organisms with the specific "H" antigens "a", "c", "k" and "r" are not frequent in America. *S. cholerae-suis* occurs in this hemisphere as the monophasic non-specific variety, called *kunzendorf*. On the other hand, salmonellae with the specific somatic antigen "y" are not often encountered in other parts of the world. With the exception of *S. paratyphi A*, the salmonellae enumerated here as organisms with antigens infrequently encountered are biphasic, *i.e.*, they possess also a non-specific antigen which is common for several salmonella types.

TABLE 2
Salmonellae used for the preparation of the polyvalent "O" antigen

ANTIGEN	SALMONELLA USED FOR ITS PREPARATION
(I), IV, (V), XII	<i>S. paratyphi B</i> var. <i>java</i>
VI, VII	<i>S. montevideo</i>
VI, VIII	<i>S. newport</i>
IX, XII	<i>S. typhosa</i>
III, X, XXVI	<i>S. london</i>

The partial antigens "g" and "m" occur in a number of salmonellae, as well as the group "e, h" and "l, v". The specific antigens of *S. paratyphi A*, *S. paratyphi B*, *S. typhimurium* and *S. typhosa* are important because of the frequent occurrence of these organisms. The specific "H" agglutinin, therefore, will have to contain the following antigens and antigenic groups: "a", "b", "d", "e, h", "l, v", "g, m, s . . .".

Eight *Salmonella* strains were selected to supply the enumerated antigens. Table 2 shows the organisms used for the preparation of the polyvalent "O", table 3, the strains necessary for the preparation of the polyvalent "H" antigen. The latter table relates also the anti-sera which are necessary to produce proper antigenic phases.

TECHNICS

In the preparation of the polyvalent antigens, the method of Welch and Stuart (1936) was used, with some modifications.

Polyvalent Salmonella "O" agglutinin

The cultures enumerated in table 2 are inoculated (separately) into tubes containing one per cent Tryptone broth with one-half per cent sodium chloride. After 16-18 hours incubation one ml. from each tube is seeded to the surface

of bacto-Trypose agar in a Kolle flask and evenly spread. After 48 hours incubation the growth is washed off with about 10 ml. absolute ethanol per flask and filtered through coarse cheese cloth. The filtrates are collected into centrifuge tubes and kept in the ice box for 48 hours, under frequent shaking. They are then centrifuged for one hour at 1,500 R.P.M., the supernatant fluid decanted and substituted with 12 per cent sodium chloride using 7 ml. for each ml. of packed bacterial cells. Equal amounts of antigens representing each *Salmonella* strain used for the preparation of the polyvalent agglutinin are mixed, the total volume adjusted so that the density of the suspension equals Barium Sulfate Standard No. 10. Finally, enough 1 per cent crystal violet is added to make the final concentration of the dye 1:40,000. The antigen is stored in the ice box.

TABLE 3
Salmonellae used for the preparation of the polyvalent "H" antigen

ANTIGEN	SALMONELLA CULTURED	SALMONELLA SERUM USED TO SUPPRESS UNDESIRABLE PHASE
a	<i>S. paratyphi A</i>	none
b	<i>S. paratyphi B</i> var. <i>java</i>	none
d	<i>S. typhosa</i>	none
g,m,s . . .	<i>S. montevideo</i>	none
i	<i>S. typhi-murium</i>	<i>S. newport</i>
e,h	<i>S. newport</i>	<i>S. typhi-murium</i>
l,v	<i>S. london</i>	<i>S. anatum</i>
1,2	<i>S. newport</i>	<i>S. anatum</i>
1,5	<i>S. cholerae-suis</i> var. <i>kunzendorf</i>	none
1,4,6	<i>S. london</i>	<i>S. panama</i>

Polyvalent Salmonella "H" agglutinin

Table 3 shows the organisms used for the preparation of this antigen. "H" antigens from monophasic cultures (*S. paratyphi A*, *S. paratyphi B* var. *java*, *S. cholerae-suis* var. *kunzendorf*, *S. montevideo* and *S. typhosa*) are prepared in the following way:

Inoculate Tryptone broth tubes and Kolle flasks as when preparing "O" antigen. After 24 hours wash the growth with about 10 to 15 ml. of 12 per cent sodium chloride containing 0.5 per cent formol. Filter through loose cheese cloth and collect into centrifuge tubes. Let stand for 48 hours in the ice box, then centrifuge at 1,500 R.P.M. for one to two hours. Leave about 1 ml. supernatant fluid for each 5 ml. cells.

Biphasic cultures (*S. typhi-murium*, *S. newport* and *S. london*) have to be present in proper phases. Therefore, before they are seeded to Kolle flasks, they are cultivated in the presence of those *Salmonella* antisera which suppress the undesired phases. One loopful of the respective serum added to the 7 to 10 ml. of Tryptone broth will sufficiently suppress one phase and "bring out" the other, so that the Kolle flasks will show growth rich in that phase which

is not suppressed. Table 3 lists the sera necessary for the preparation of such antigens. The growth from the Kolle flasks is collected and treated as that from monophasic cultures for the preparation of "H" antigens.

Equal amounts of the "H" antigens each representing one phase, are mixed, the total volume adjusted so that the density of the suspension equals Barium Sulfate Standard No. 10. Finally, enough one per cent malachite green is added to make the final concentration of the dye 1:20,000. The antigen is stored in the ice box.

The rapid slide agglutination test

(a) Exclusion test.

A glass depression slide with two (or more) excavations is used. With a Kahn pipette, 0.04 ml. serum is pipetted into each of two excavations. To one of the drops 0.03 ml. of the "O" to the other 0.03 ml. of the "H" antigen is added and thoroughly mixed with a wooden applicator. The slide is rocked for one minute and the results read with the naked eye. Only a 4 + reaction (complete agglutination) is reported as positive. A positive slide test involving 0.04 ml. serum and 0.03 ml. antigen is equivalent to a positive tube agglutination test with 1:40 dilution of the serum.

Sera showing no agglutination in this test are considered negative. Those which show complete agglutination with at least one antigen are either reported as positive or subjected to serial testing. While the second method is more recommended for clinical use, the first procedure will save much time and material in surveys.

(b) Serial testing.

A glass depression slide, preferably with rows of 4 excavations, is used. Into two rows of excavations 0.04, 0.02, 0.01 and 0.005 ml. of serum, respectively, are delivered. "O" antigen is added to the first, "H" antigen to the second row, using 0.03 ml. of antigen for each drop of serum. The dilutions are equivalent to 1:40, 1:80, 1:160 and 1:320 of the routine tube agglutination test. The results are read after proper mixing and rocking the slide for one minute. Only 4 + (complete agglutination) is considered positive. The results are reported as those of any other serial agglutination test.

EVALUATION

Before the above described *Salmonella* antigens were devised, several other combinations were tested. One of them (Felsenfeld, 1946) was composed of 9 "O" and 14 "H" antigens, which correspond to one or more of the antigens of 95 to 98 per cent of the salmonellae encountered in the United States. Tests with these agglutinogens have to be reserved for North America and her large laboratories because of the difficulties in the reading of the results. Using the polyvalent antigens described in this paper, one faces the usual obstacles encountered in the serodiagnosis of diseases caused by Enterobacteriaceae.

As it was pointed out by Bornstein (1943), the agglutinating antibodies in salmonellosis are not always formed in quantities sufficient for their laboratory

detection. Their antibody production is slow, chiefly in *Salmonella enteritis*, or may be absent.

Cross-reactions occur very often. This circumstance, however, does not represent a disturbing factor when polyvalent antigens are used.

One of the most precarious problems in the laboratory diagnosis of salmonellosis is the evaluation of positive agglutination tests in recently immunized persons. "Anamnestic" reactions are not rare. A person who was immunized with the T.A.B. vaccine or had a *Salmonella* infection, may show an increase of agglutinating antibodies in the serum during a febrile disease.

TABLE 4
Results of agglutination experiments

GROUP	NO. EXAM- INED	POSITIVE REACTION WITH							
		Polyvalent antigens				Typhoid, paratyphoid A and B commercial antigens			
		1:40 or less	1:80	1:160	1:320 or more	1:40 or less	1:80	1:160	1:320 or more
Causative organism of infection:									
<i>S. typhosa</i>	3			1	2			1	2
<i>S. paratyphi B</i>	7		2	2	3		4	1	2
<i>S. typhi-murium</i>	23	3	10	5	5	17	1	5	
<i>S. cholerae-suis</i>	4	1		2	1	3		1	
<i>S. oranienburg</i>	6		2	1	3	5	1		
<i>S. montevideo</i>	7	1	2	3	1	7			
<i>S. anatum</i>	2	1	1			2			
<i>S. panama</i>	4		1	2	1	3		1	
<i>S. senftenberg</i>	1			1		1			
<i>S. give</i>	2		1	1		2			
<i>S. newport</i>	1			1		1			
	60	6	19	19	16	41	6	9	4
Immunized persons T.A.B.....	52	4	18	11	19	5	20	14	13
Febrile diseases, cystitis, pyclitis, etc.....	47	29	10	4	4	34	6	4	3
Non-immunized persons with neg. story.....	56	51	3	1	1	54	1		1

Finally, many Enterobacteriaceae contain *Salmonella* antigens. In infections caused by such organisms, particularly colon-paracolon bacteria having *Salmonella* antigens, positive serologic tests may occur with *Salmonella* agglutinogens.

Table 4 shows the results of tests with polyvalent *Salmonella* "O" and "H" antigens compared with the outcome of agglutination reactions in the same persons using reliable commercial typhoid, paratyphoid A and paratyphoid B antigens. In all instances the final agglutination titres given in the tabulation show the highest titer observed.

6 of the 60 *Salmonella* infections, i.e., 10 per cent, did not give a positive test with polyvalent "O" and "H" antigens, while 41 of them, i.e., 66.7 per cent, were not revealed by the use of the routine typhoid, paratyphoid A and B sero-diagnostic set. On the other hand, more non-specific reactions were observed with the polyvalent antigens. These non-specific reactions, however, did not show an increasing titer when the test was repeated one or two weeks later. It has to be concluded, therefore, that the polyvalent antigens are more reliable in the serodiagnosis of salmonellosis, if in case of doubt the test is repeated.

SUMMARY

Polyvalent "O" and "H" antigens for the serodiagnosis of salmonellosis were described. These antigens are composed of *Salmonella* agglutinogens corresponding to the antigens of most salmonellae found under different climates. They were tested in 60 cases of salmonellosis, 52 persons immunized with T. A. B. vaccine, 47 patients with diseases often giving non-specific positive Widal tests and 56 persons who were not immunized nor had a story of salmonellosis. The antigens were more reliable for the diagnosis of salmonellosis than the routine set of typhoid, paratyphoid A and B antigens.

ACKNOWLEDGEMENT

The author is indebted to Paul H. Hletko, M.D., Chief Medical Officer of the Illinois Department of Public Welfare, for the permission to use some of the data collected during his work in Illinois State Hospitals in this connection.

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THE GEOGRAPHICAL DISTRIBUTION OF SHIGELLA¹

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Any discussion of the types contemplated in this paper precludes the necessity of clarifying the terminology involved. Neter's (1942) classification of shigellae has been generally accepted in America. His schema, therefore, is utilized in the present article. Neter divides shigellae found in man into three groups:

1. A mannitol-negative, lactose-negative group, which includes *Sh. dysenteriae* Shiga and *Sh. ambigua* Schmitz. *Sh. newcastle* Clayton-Warren is added to this group, in spite of the fact that this organism is slightly motile and, under favorable conditions, forms some gas. Both of these qualities are not observed in other shigellae.

2. A mannitol-positive, lactose-negative group, to which belong *Sh. paradysenteriae* Flexner and Boyd, *Sh. alkalescens* and *Sh. gallinarum*. The latter, however, is better classified as a *Salmonella*, due to its antigenic structure.

3. A mannitol-positive, lactose-positive group, into which *Sh. sonnei* and *Sh. dispar* are placed.

A variant of *Sh. newcastle* called the Manchester bacillus, is a strong gas producer. Both are antigenically but not biochemically identical with *Sh. paradysenteriae* VI (Boyd 88).

Several new strains belonging to this group were described during the last few years. Wheeler and Stuart (1946) discussed these strains and found that only the so-called Sachs strains (1944) represent new but very rarely encountered types.

Sh. paradysenteriae includes the strains described by Flexner, Boyd, Strong and Hiss-Russell. The nomenclature of Andrewes and Inman which was in general use is being replaced by the terminology of Boyd, Wheeler (1944a, 1944b, 1944c) and Weil (1943). These systems substitute Roman numerals in place of the letters used by Andrewes and Inman and the letters and arabic numerals employed by Boyd. Wheeler (1944b, 1944c) excluded the formerly accepted "X" and "Y" types as rough strains and does not list types "WX" and "VZ" of Andrewes and Inman, while Weil and Farsetta (1946) consider strain "WX" (II.VII in Weil's classification) a valid, existing type. Francis (1946) described two additional *Sh. paradysenteriae* strains. Heller and Wilson (1946) added *Sh. clousae*, which is, however, a transitional form, as indicated by its action on milk and the reduction of trimethylamine oxide. Table 1. presents the nomenclatures used by most of the present writers. Within the frame of this classification we are listing Sachs's organism Q 902 as a *Sh. ambigua*, because of its biochemical properties.

Due to the activities of Army centers, many reports from hitherto unstudied

¹ Read at the Annual Meeting of the American Society of Tropical Medicine, November 4-7, 1946, Miami, Fla.

areas have been added to the information furnished by the established *Salmonella-Shigella* typing centers. From these collective efforts it has been possible to draw almost world-wide conclusions as to the distribution of dysentery bacilli.

1. Mannitol-negative, lactose-negative shigellae.

One of the least common of the *Shigella* strains reported in the United States is *Shigella dysenteriae* Shiga, having been described recently only from the

TABLE 1
Terminology of Shigella

NEW DESIGNATION	FORMER DESIGNATIONS
<i>Sh. dysenteriae</i> Shiga	Shiga-Kruse bacillus. "True" dysentery bacillus
<i>Sh. dysenteriae</i> Sachs Q 454	Sachs type Q 454
<i>Sh. dysenteriae</i> Sachs Q 771	<i>Sh. arabinotarda</i> A, Giber 8524, Sachs type Q 771
<i>Sh. dysenteriae</i> Sachs Q 1030	Sachs type Q 1030
<i>Sh. dysenteriae</i> Sachs Q 1167	<i>Sh. arabinotarda</i> B, Sachs type Q 1167
<i>Sh. ambigua</i> Schmitz	Schmitz bacillus
<i>Sh. ambigua</i> Sachs Q 902	Sachs type Q 902
<i>Sh. newcastle</i>	<i>Sh. newcastle</i> , Clayton-Warren, Manchester
<i>Sh. paradysenteriae</i> Flexner I	Flexner V
<i>Sh. paradysenteriae</i> Flexner II	Flexner W
<i>Sh. paradysenteriae</i> Flexner III	Flexner Z, Strong bacillus
<i>Sh. paradysenteriae</i> Flexner IV	Boyd 103
<i>Sh. paradysenteriae</i> Flexner V	Boyd P 119
<i>Sh. paradysenteriae</i> Flexner VI	Boyd 88
<i>Sh. paradysenteriae</i> Flexner VII	Described by Francis (1946)
<i>Sh. paradysenteriae</i> Flexner VIII	Described by Francis (1946)
No number assigned	Flexner X
No number assigned	Flexner Y, Hiss-Russell organism
No number assigned	Cope and Kilander (1942)
<i>Sh. paradysenteriae</i> Boyd I	Boyd 170
<i>Sh. paradysenteriae</i> Boyd II	Boyd P 288
<i>Sh. paradysenteriae</i> Boyd III	Boyd D 1
<i>Sh. paradysenteriae</i> Boyd IV	Boyd P 274
<i>Sh. paradysenteriae</i> Boyd V	Boyd P 143
<i>Sh. paradysenteriae</i> Boyd VI	Boyd D 19
<i>Sh. alkalescens</i>	Same
<i>Sh. sonnei</i>	Same, Kruse "E" bacillus, <i>Sh. dispar</i>
<i>Sh. dispar</i>	<i>Sh. ceylonensis</i> , <i>Sh. madampensis</i> .

New Orleans area by Silverman and Friedrichs (1943) and from Detroit by Sandweiss (1944). Immigrants from southern countries are suspected as sources of the infection in Michigan. With the increased traffic, there is a growing possibility that this important dysentery microorganism will find its way into other regions, but such events have not as yet been reported in the literature. In the analysis of the geographic distribution of *Sh. dysenteriae* it has to be kept in mind that the primary isolation of this organism may cause techni-

cal difficulties. The highly selective plates often exclusively used for the plating of stools are not favorable for the growth of *Sh. dysenteriae*. Delayed examination of the stools strongly decreases the number of positive findings. It is, therefore, doubtful as to whether the organism is as rare in the Americas as the statistics indicate.

Mendez Silva (1943) and J6o (1944) found *Sh. dysenteriae* Shiga frequently in Europe, and Berge and Fauconnier (1941) and Boyd (1946) had similar findings in Africa and East Asia. J. B. Nelson *et al.* (1946) encountered it in India, and Fortune and Ferris (1945) in New Guinea. Esseveld *et al.* (1941) rarely discovered it among the natives of the Netherlands Indies.

Another *Shigella* strain which is rare in the population of the United States is *Sh. ambigua*. We find it chiefly in veterans returning from the Pacific area. Numerous cases were also isolated in Midwestern State Hospitals (Felsenfeld and Young, 1944) where this strain was introduced by a carrier. Hormaeche *et al.* (1943) found *Sh. ambigua* frequently in South America, Mendez Silva (1943) on the Iberian peninsula and Berge and Fauconnier (1941), Martin (1946) and Boyd (1946) in North Africa and the Near East. In Hungary (J6o, 1944) and New Guinea (Fortune and Ferris, 1945) it is less frequent than *Sh. dysenteriae*. It is also present in India.

The Sachs strains are too rare to be included into this discussion.

Sh. newcastle strains are now discovered more frequently than heretofore. Hardy and Watt (1945) found it in Georgia, New Mexico and New York. Rubenstein and Philips (1944) described a mild outbreak in the West. Hormaeche *et al.* (1943) and Leite Ribeiro (1946) frequently encountered this strain in South America. Many of the European countries suffer from *Sh. newcastle* dysentery. Included among them are Holland and Hungary, according to the reports of Westermann (1944) and J6o (1943). Its presence in the German army caused its spread to the countries under occupation. *Sh. newcastle* was found frequently in the Netherlands Indies by Esseveld *et al.* (1941).

2. Mannitol-positive, lactose-negative shigellae.

Wheeler (1944a) encountered many *Sh. paradyenteriae* Flexner I and VI types in New England. Type Flexner III was frequent in his statistics, because of an institutional outbreak. Hardy and Watt (1945) encountered mostly *Sh. paradyenteriae* Flexner I and II in mental hospitals, while Felsenfeld and Young isolated most frequently types I, III, IV and VI of *Sh. paradyenteriae* Flexner in similar institutions. C. T. Nelson *et al.* (1946) described many *Sh. dysenteriae* II, VI and "X" strains in the Southeastern states, while Fulton and Curtis (1946) found types II and IV of this organism prevalent in Texas. Zozaya and Villanueva (1943) in Mexico isolated many *Sh. paradyenteriae* Flexner II and III strains, as did Yáñez (1943) in Spain and Mendez Silva (1943) in Portugal. Elrod and Wormus (1946) found mostly *Sh. paradyenteriae* Flexner II and VI in Eastern France and Widman chiefly the "Y" type in Eastern Europe. J. B. Nelson *et al.* (1946) described many Flexner I, II, IV and VI strains in India. Kessel *et al.* (1945) in California, Kuhns (1943) in Georgia, Hormaeche *et al.* (1943) in South America, J6o (1944) in Hungary, Boyd (1946) in the Near East and Fortune and Ferris (1945) in New Guinea, found *Sh. para-*

dysenteriae Flexner the predominant cause of bacillary dysentery. According to the above statistics, types *Sh. paradysenteriae* Flexner I and IV are more circumscribed in distribution than types II and III. The former are encountered more frequently than other strains of *Sh. paradysenteriae* in the Americas and in India.

Sufficient numbers of cases and outbreaks of the less common *Sh. paradysenteriae* Flexner and Boyd types, including the new strains recently described, have not been reported.

Sh. alkalescens is universal in its occurrence. It is very frequent in children and in mental institutions.

3. Mannitol-positive, lactose-positive shigellae.

Originally *Sh. sonnei* was described from the Scandinavian countries. It was formerly very rarely reported in the United States but has since become wide spread in its distribution. This is true not only for the United States but also throughout the other areas included in this study. Wheeler (1944a) found many strains in New England, Hardy and Watt (1945) and Felsenfeld and Young (1944) in mental institutions, C. T. Nelson *et al.* (1946) in the South-eastern states, Kuhns (1943) in the U. S. Army, Kessel *et al.* (1945) in California, Fulton and Curtis (1946) in Texas, Macumber (1942) in Panama, Zozaya and Villanueva (1943) in Mexico and Steuer and Groeger (1940) in Poland. J6o (1943) in Hungary and Roelcke and Neuberg (1941) in Germany isolated *Sh. sonnei* mainly from children. This organism is often encountered, but is not the most frequent cause of bacillary dysentery in South America (Hormaeche *et al.* 1943), in Venezuela by Briceño-Iragorri (1943) and Holland (Westermann, 1944). It was found in France by Elrod and Wormus (1946), in the Near East by Boyd (1946) and New Guinea by Fortune and Ferris (1945).

Sh. sonnei is a common cause of food poisoning. Recently Finlayson (1944) described a milk-borne outbreak. Hailwood (1944) traced an epidemic to a cook. Green and McLeod (1943) found the water used to wash bottles in a dairy to be carrying *Sh. sonnei* infection. The disease caused by *Sh. sonnei* is a mild diarrhea rather than the typical textbook picture of bacillary dysentery. The stools contain blood only exceptionally. The clinical picture, except in small children and old people, is usually a self-limited diarrhea without much alteration of the general health. Many cases therefore escape the attention of the proper authorities, because they are never brought to the attention of a physician, being considered "a simple diarrhea" or a "food upset". *Sh. sonnei*, however, is a very frequent cause of the so-called "summer diarrhea" and of "infantile diarrhea." Its laboratory diagnosis seldom causes technical difficulties, because most of the Sonne strains grow well on the routine media.

Sh. dispar is considered by many authors as a non-pathogenic organism, and is seldom included in survey reports. The effect of this conception on the literature makes it impossible to discuss the epidemiology.

DISCUSSION

The geographic survey of shigellae has shown that the most important strains are *Sh. dysenteriae* Shiga, *Sh. ambigua* Schmitz, *Sh. newcastle*, *Sh. paradysenteriae*

Flexner I, II, III, IV and VI and *Sh. sonnei*. The exact diagnosis of the type of the causative strain is not of paramount importance in every case of bacillary dysentery when only the therapeutic aspects are kept in mind, because the treatment of patients who do not show toxic symptoms is restricted to diet, high fluid intake and sulfonamides, the serotherapy being reserved for toxic cases, mostly evoked by *Sh. dysenteriae* Shiga. From the epidemiologic and laboratory point of view, however, the typing of the shigellae is a *conditio sine qua non*. The source of the infection cannot be detected without the knowledge of the type which caused the infection. In the laboratory, it is important to use plating media and diagnostic sera which are favorable for the detection of the most frequent and most important strains. Many *Sh. dysenteriae* Shiga do not grow on selective media on which *Sh. paradysenteriae* and *Sh. sonnei* are easily cultured. It is necessary, therefore, to include non-selective plates, as McConkey's medium, Eosin-methylene blue agar, Leifson's desoxycholate plate or the D.E.C. medium of Panja and Ghosh in the series used for the culturing of the stools. The use of such plates will also aid in the isolation of the rare fastidious *Sh. sonnei* strains.

In order to facilitate the differentiation of microorganisms, polyvalent sera are used for the group-diagnosis. Two sera are recommended: a polyvalent *Salmonella* serum which includes also *E. typhosa* agglutinating antibodies (Felsenfeld and Young, 1945) and a polyvalent *Shigella* serum. If agglutination occurs, a preliminary report is issued which is followed in due time by the exact diagnosis of the strain or strains involved. Only colonies grown on non-selective media can be used for testing directly with polyvalent sera, because selective media inhibit serologic reactions.

The discussion of the geography of *Shigella* had of necessity to be based on recent statistics due to the former lack of suitable classification and serologic typing technics. Many of the older statistics were inadequate in this respect. It is to be hoped that typing will be carried out more and more extensively by future workers to enable further reaching conclusions to be drawn concerning the genus *Shigella*.

CONCLUSIONS

In conclusion the following points should be emphasized:

1. The scarcity of reports on *Sh. dysenteriae* Shiga in the Americas.
2. The increasing incidence of *Sh. newcastle*, mainly in America and Europe.
3. The greater localization of *Sh. paradysenteriae* Flexner I and IV to America and India and the cosmopolitan distribution of types II and III.
4. The widespread distribution of *Sh. sonnei*, mainly in North and Central America and Eastern Europe.
5. The necessity of proper laboratory aids for the type diagnosis of shigellae.

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ATTEMPTS TO CULTIVATE THE MOSQUITO PHASE OF *PLASMODIUM RELICTUM*

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Although a number of attempts have been made to cultivate malarial parasites outside of the body of the host, experiments have been limited almost entirely to the asexual cycle, which is found in the vertebrate host. Bass and Johns (1912), who were among the first to report the successful cultivation of *Plasmodium* from man suggested the possibility of cultivating the sexual cycle *in vitro* also, but apparently did not follow up this suggestion.

The usual course of *in vitro* cultivation of the erythrocytic cycle from the vertebrate host has been a persistence in the blood for several days, followed by a dying out of the culture after at most an apparent multiplication for three or four generations. Recently, however, E. G. Ball *et al* (1945), McKee *et al* (1946) have reported the multiplication *in vitro* for at least six generations of *Plasmodium knowlesi* from the monkey at rates comparable to those in the body.

In 1939, Hegner and Wolfson, using tissues removed from infected canaries, were able to demonstrate the persistence of *Plasmodium cathemeium* in tissues cultured for fifteen days, although the oldest culture infective to clean birds was one of eight days. Hawking (1945) cultivated the exo-erythrocytic stages of *Plasmodium gallinaceum* in tissue culture for eighty-nine days. At the end of this time, the parasites were still capable of infecting clean chicks.

The use of insect tissue cultures, or of what are perhaps better called organ cultures, for *in vitro* studies of the insect phases of parasitic organisms was reported by Trager (1937, 1938), who obtained in this way (1937) some development of *Nosema* in silkworm ovarian tube cells, and in (1938) a titre increase of 100,000 of the virus of equine encephalomyelitis in mosquito tissues in twenty-eight days.

In the experiments reported here, mosquitoes belonging to the species *Culex tarsalis*, which had metamorphosed in the laboratory, were allowed to bite canaries infected with *Plasmodium relictum*-Strain 1P1-1,¹ 81.6 per cent of *C. tarsalis* biting parasitized birds became infected.

On various days after biting, the mosquitoes were killed and the stomachs transferred to culture media for the study of further development of the parasite. Because it is difficult to identify the younger stages in the stomach *in vitro*, the majority of the mosquitoes were killed from six to ten days after biting when the oocysts were easily visible on the stomach wall.

If the mosquitoes were kept at a low temperature (18°C. in a constant temperature room), they could be deprived of food and water for forty to fifty hours with practically no deaths. After this interval, the majority of stomachs were free of all or of nearly all bacterial or fungal contaminants. Hinman (1930)

¹ This strain was obtained January 27, 1945, from Professor C. G. Huff of the University of Chicago and has been maintained since then by intravenous transfer.

noted that in larval culicids, bacteria were not commonly found in the gut in nature. The mosquitoes were killed and their external surface sterilized by dropping them into a solution of hexylresorcinol, or occasionally aqueous iodine $\frac{1}{1000}$, for five minutes. They were then transferred to a sterile chamber and all further work carried on aseptically.

The following procedure was found to be the most satisfactory one. In the sterile chamber, the mosquito was first washed in a large drop of sterile Ringer's Solution on a flat glass slide. It was then transferred to a second drop of Ringer's on another flat slide and the mid- and hind-gut with part or all of the oesophagus removed from the insect through the posterior part of the abdomen. The other parts of the digestive tract were then carefully dissected away from the stomach. Usually, it was not practical to remove all of the Malpighian tubules, and in some cases, the anterior part of the hind-gut was left attached intentionally. Since the latter is contractile, it served as an objective means of determining if this part of the preparation was still alive. In general, however, it is preferable to trim off as much of the fore- and hind-gut as possible since these are the principal areas of bacterial contamination. The cleaned stomach was then transferred to a sterile #2 (3 mm.) eye spoon and carried over to a depression slide filled with sterile Ringer's. Here it was agitated for two minutes and then transferred by the eye spoon successively to six sterile depression slides filled with sterile Ringer's Solution and agitated for one or two minutes in each slide. From the last slide, the stomach was transferred by a sterile wide-mouthed pipette to the culture medium in a Carrel flask.

Various culture media have been employed. Because of the success attained by Trager (1938) in cultivating the virus of equine encephalomyelitis in mosquito tissues, the medium employed by him was the one first used. This is essentially a buffered mixture of simple salts, glucose, egg digest, and plasm. Both rabbit and chicken plasm were used, the former being employed more frequently. In some instances, no plasm was added, leaving a completely fluid medium. When no growth was apparent in the oocysts with Trager's original medium, the proportion of glucose was doubled since Fulton (1939), Wendel (1943), and others had shown that *Plasmodium knowlesi* utilized glucose rapidly *in vitro* and that the addition of glucose to infected blood *in vitro* increased the length of time that the parasites remained viable (Fulton, 1939). Silverman, Ceithaml, Taliaferro and Evans (1944) found a similar increase in glucose utilization in erythrocytes infected with *P. gallinaceum*.

Another type of medium used was an entirely synthetic, so-called complete medium. Media of this sort have been employed successfully for the bacteria-free cultivation of *Tetrahymena geleii*, a fresh-water ciliate. Cultures of infected mosquito stomachs were tested in the original medium of Kidder and Dewey (1945) as well as in various modifications of this medium which had been made by Professor M. S. Dunn of the Chemistry Department of this institution. All of the latter had already proved to be very successful for the cultivation of *T. geleii*. White (1946) has demonstrated that completely synthetic media can be used in the culture of metazoan tissues.

Synthetic media were used either with an agar base or in liquid form. In

some instances, they were used in combination with plasm. In some of these series also, the proportion of glucose was increased.

In some cases, fresh medium was added daily and the old culture medium removed. Usually, however, the medium was not changed during the course of the experiment. Approximately 2 cc. of medium was used for each stomach.

The gas content of the culture flasks was altered in various ways. The type of mixture employed of course influenced in turn the hydrogen-ion concentration of the medium. If the flask was closed with only a cotton plug after the implantation of the stomach tissue, the medium soon became too acid for the culture to survive. The flasks, therefore, were sealed either with paraffined cork stoppers or with rubber plugs, which were then coated with ambroid. The volume of tissue in a Carrel flask was very minute compared with the amount of gas or air available. Nevertheless, it was felt advisable to change the gas or air content daily. With atmospheric air, the flasks were opened daily for from ten to fifteen minutes; during this time, the flask contents were protected by inserting a sterile cotton plug into the neck of each flask.

At the termination of an experiment, hydrogen-ion determinations were made with a glass electrode. To check pH changes during the course of an experiment, sterile phenol red was added so as to make a concentration of 0.005 per cent and the color compared with those in a series of flasks with known pH readings. No deleterious effect was observed on the cultures from the addition of the indicator.

Some of the media were noticeably alkaline when they were first used. These were gassed with a sterile mixture of 76 per cent N_2 , 3 per cent CO_2 , and 21 per cent O_2 (Parker, 1938) until they showed a neutral reaction. In many instances, the medium containing the tissue became more acid as the experiment progressed. In these cases, it was necessary to control the pH by gassing the medium daily with sterile CO_2 -free air.

Despite the exceedingly large volume of air or gas available to the very small piece of tissue in the flask and the daily change of air, it was possible that failure to grow was due to asphyxiation of the host tissue or of the parasite. Wendel (1943) working with *Plasmodium knowlesi*, found an increased oxygen consumption of 300 times, and Silverman, Ceithaml, Taliaferro and Evans (1944), with *P. gallinaceum*, an increase of 70 times for parasitized erythrocytes as compared with normal ones. Consequently, in one series, a stream of moist sterile air was passed through the flask continuously for as long as seven days.

Since a certain percentage of the cultures were lost by the growth of contaminating bacteria or molds, attempts were made to keep these under control by the addition of up to 100-200 units of penicillin per 1 cc. of medium. Curran and Evans (1945) showed that penicillin was an effective bacterial sporocide in low concentrations, while Hawking (1945) found that low concentrations did not interfere with the growth of exo-erythrocytic stages of *Plasmodium gallinaceum in vitro*. Because of the fairly rapid breakdown of penicillin at room temperature, it is necessary to add fresh penicillin every day or every other day. Penicillin was effective in controlling light contamination.

In the work of E. G. Ball *et al.* (1945), it was demonstrated that some rocker

type of culture was necessary for the cultivation of the asexual phase of *Plasmodium knowlesi* in erythrocytes, although Hawking (1945) did not find rocking necessary to cultivate the exo-erythrocytic stages of *P. gallinaceum*. In some of



FIG. 1. STOMACH OF *CULISETA INCIDENS*. 4-DAY CULTURE. $\times 610$

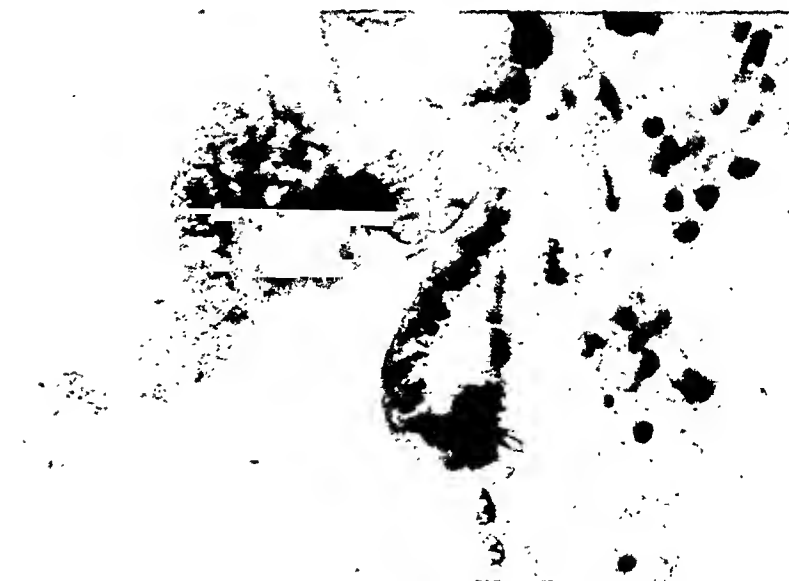


FIG. 2. STOMACH OF *CULISETA INCIDENS*. 11-DAY CULTURE. $\times 700$

the present series of experiments, a continuous washing of the cysts and of the mosquitoes' stomachs was obtained by attaching the flasks to the blades of a "windmill," which rotated at an angle of about 30 degrees from horizontal.

In order to discover any growth or development of the oocysts, camera lucida drawings of representative cysts were made daily from those stomachs held in

place in plasm or agar. In the case of those kept in liquid media, the stomachs were manipulated until they were stranded on the bottom of an inverted Carrel flask, and drawings were made daily of several cysts from one particular area of



FIG. 3. STOMACH OF *CULEX TARSALIS*, SHOWING OOCYSTS OF *PLASMODIUM RELICTUM*, 6-DAY CULTURE. MOSQUITO INFECTED SEVEN DAYS BEFORE CULTURE. $\times 510$



FIG. 4. STOMACH OF *CULEX TARSALIS*, SHOWING OOCYSTS OF *PLASMODIUM RELICTUM* FROM MOSQUITO INFECTED 10 DAYS PREVIOUSLY. NOT CULTURED. $\times 340$

the stomach. Developmental stages of the cultured oocysts were compared with normally developing oocysts from mosquitoes biting at the same time. At the end of each experiment, the stomachs were fixed and usually sectioned.

The mosquito tissue showed contractility in the hind-gut for as long as seven days in culture. Furthermore, sections of mosquito stomachs showed apparently normal nuclear structure for as long as ten days (figs. 1, 2). This period would be long enough to show a considerable development of the cyst since the normal cycle in the mosquito is about two weeks. The cultures were maintained at room temperature, which never dropped below 20°C. Since cysts about one week old were used in most cases, a large part of them should have reached maturity.

Despite the persistence of living mosquito tissue *in vitro*, the cysts of *Plasmodium relictum* in this tissue did not increase in size nor did they develop further internally as indicated by an increase in the number of nuclei. On the other hand, sections of cultured oocysts indicate that they persisted *in vitro*, apparently unchanged cytologically for as long as six days without undergoing further development (figs. 3, 4).

This is believed to be the first report of an attempt to cultivate the sporogonic stages of *Plasmodium in vitro*. The results indicate that host tissue as well as the oocysts can be maintained in culture for six or seven days. But with none of the techniques outlined above: alterations of the media, modification of the gas content of the flasks, continuous aeration, or modified rocker cultures was there any apparent development of the oocysts.

SUMMARY

In an endeavor to bring about the development of the sporogonic cycle of *Plasmodium in vitro*, attempts have been made to culture the oocysts of *P. relictum*, strain 1P1-1, on the stomachs of *Cules tarsalis*. A number of media, including completely synthetic ones were tested. Various mixtures of gases were introduced into the flasks, in some cases continuous aeration was employed, in others a modified rocker type culture was used. Mosquito tissue remained alive with hind-gut contraction for at least seven days; oocysts of *P. relictum* persisted apparently unaltered for six days, but no development of the oocysts could be demonstrated.

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CLINICAL STANDARDIZATION OF PAMAQUIN (PLASMOCHIN) IN MOSQUITO-INDUCED *VIVAX* MALARIA, CHESSON STRAIN

A PRELIMINARY REPORT¹

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Pamaquin (plasmochin, plasmoquine, praequin) has been recognized for many years as an antimalarial drug having unusual properties. Sinton and Bird (1) in India in the late 1920's were among the first to test the efficacy of this newly produced synthetic drug. They made studies on European soldiers with naturally acquired *Plasmodium vivax* (benign tertian) malaria. The administration of pamaquin along with quinine for 28 days of continuous treatment resulted in the prevention of relapses in 18 of 20 subjects, two being lost for follow-up. Control cases treated with quinine only, suffered a relapse rate of 77 per cent. The pamaquin dosage was 0.1 gram a day. Since it was not reported what salt of pamaquin was used, the weight of pamaquin base is not known. Interruption of the therapy and the administration of pamaquin without quinine were less effective. Sinton, Smith and Pottinger (2) subsequently found that periods of treatment shorter than 28 days and dosages as small as 0.04 gm. of the salt a day were satisfactory.

Piebenga, in 1930, (3) treated *vivax* malaria acquired naturally in the Netherlands, with a 14-day course of pamaquin and quinine. Of 67 patients treated, only one relapsed. Quinine-treated control subjects suffered a 60-70 per cent relapse rate.

It was the conclusion of the Malaria Commission of the League of Nations (4) that a combination of pamaquin and quinine was "most efficacious" in reducing the number of relapses in benign tertian malaria. In spite of these early favorable reports, however, pamaquin fell into disrepute because of its

¹ This investigation was carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. The work was planned in conjunction with the Panel on Clinical Testing of Antimalarials of the Board for the Coordination of Malarial Studies. This work was further aided by the participation of Army Medical Officers assigned to the project by The Surgeon General, U. S. Army.

Through a cooperative arrangement between Professor Clay G. Huff and Dr. Frederick Coultsen, Department of Bacteriology and Parasitology, and the Malarial Research Unit, Department of Medicine, the former group bred *Anopheles quadrimaculatus* mosquitoes, supervised their infection and the inoculation of volunteers, and determined the intensity of infection in the salivary glands of the mosquitoes. The latter group assumed the responsibility for clinical care of patients studied by both groups.

² Captain, M.C., A.U.S.

toxicity and in 1943, its use in the armed services of the United States was discontinued (5).

The precise definition of the activity and toxicity of pamaquin under carefully controlled conditions was undertaken in 1944 by several laboratories under the direction of the Board for the Coordination of Malarial Studies in order to develop standards for the appraisal of its analogues. A secondary objective was the reinvestigation of pamaquin as a drug clinically useful in itself, even though its toxicity made the accomplishment of this objective unlikely. These studies were begun by Feldman and his coworkers (6) in domestic strains and continued by Berliner and his coworkers (7) in the Chesson strain of *vivax* malaria. The latter group demonstrated that pamaquin, when given concurrently with quinine in the maximum dose tolerated in man, 90 milligrams of base a day, for two weeks, cured 9 out of 9 volunteers with sporozoite-induced infections. A dose of 30 milligrams a day, given with quinine, or 90 milligrams a day, given without quinine failed to cure some of the subjects.

The favorable results obtained in the 90-milligram experiments prompted the Board to recommend the further exploration of pamaquin reported herein and, later, a clinical trial on military personnel, which was carried out at Moore General Hospital. There, Hayman *et al.* (8) found that only 27 milligrams of pamaquin base daily, given with quinine for two weeks, produced a precipitous drop in the relapse rate. Their patients were returned soldiers, who had been infected overseas under field conditions and had been on prolonged quinacrine suppression.

METHODS

The therapeutic testing procedures have been described in detail elsewhere (9). Briefly, the test drugs were administered to presumably susceptible white, male volunteers in the Illinois State Penitentiary (a non-endemic location), beginning promptly after the onset of primary attacks or early relapses of mosquito-induced *vivax* malaria (Chesson). Plasma drug concentrations were determined and evidence of toxicity was collected. The effect on relapse rate and on the duration of the subsequent latent period was determined by the examination of thick blood smears, which were made at frequent and regular intervals until relapse occurred.

In order to determine whether the action of quinine enhanced the effect of pamaquin, two types of studies were designed. In one, quinine was administered following the course of pamaquin, and in the other, the two drugs were given concurrently. Quinine was given as quinine dihydrochloride or sulfate in doses of 2.0 grams of the salt daily, in six equally divided doses at 4-hour intervals. Pamaquin was also given every four hours, but the doses were not always exactly equal because of the size of the pamaquin tablets. The dosages were in terms of pamaquin base, which is 45 per cent of the weight of the naphthoate salt as commercially supplied. Thus, 63 milligrams is equivalent to 140 milligrams of pamaquin naphthoate.

Nine dosage regimes were investigated; in four regimes, quinine was given for 8 days immediately after the completion of pamaquin medication, and in five regimes the two drugs were given concurrently (Table 1).

TABLE 1
Pamaquin dosage regimes

QUININE SULFATE 2.0 GM. DAILY ADMINISTERED FOR 8 DAYS FOLLOWING COURSE OF PAMAQUIN			QUININE SULFATE 2.0 GM. DAILY ADMINISTERED CONCURRENTLY WITH COURSE OF PAMAQUIN		
No. of cases	Daily dosage of pamaquin base	Duration of pamaquin treatment	No. of cases	Daily dosage of pamaquin base	Duration of pamaquin treatment
	mgm.	days		mgm.	days
1	15	14	5	15	14
5	15	28	5	15	28
7	31	14	6	31	14
5	63	14	5	45	14
			5	63	14

TABLE 2
Therapeutic effect
Pamaquin followed by 8 days of quinine

PAMAQUIN ADMINISTRATION		INDIVIDUALS RELAPSED/INDIVIDUALS TREATED (RELAPSE RATIO)	DURATION OF SUBSEQUENT LATENT PERIOD	
Base wt./day	Duration		Median	Range
mgm.	days		days	days
15	14	1/1	14	
15	28	5/5	15	12-18
31	14	7/7	14	12-54
63	14	5/5	37	31-39

RESULTS

Therapeutic Effect

Serial administration. When pamaquin was administered in doses of 15, 31 or 63 milligrams of the base daily for 14 days, followed by quinine for eight days, every patient relapsed (Table 2). The subsequent latent period was prolonged at the dosage of 63 milligrams a day. In the dosage regime of 15 milligrams a day for 28 days, followed by quinine, (second line in Table 2) not only did relapse occur promptly after the end of treatment, but parasitemia, with or without fever, recurred during the last several days of pamaquin therapy. Pamaquin and quinine, given serially at these dosages and under these conditions, failed to afford protection against relapse.

Concurrent administration. Complete protection was not obtained under the conditions of these trials when pamaquin was given in doses of 15 to 63 milligrams per day, with quinine, for 14 days, although several patients failed to relapse (Table 3). When 15 milligrams was given with quinine for 28 days

relapses also occurred. The subsequent latent period was longer after the larger doses, implying a modification of the disease short of immediate radical cure (10).

TOXICITY

The symptoms of pamaquin toxicity were anorexia, nausea, vomiting, and epigastric distress or pain. Methemoglobinemia commonly occurred, and when it exceeded 6 per cent or 7 per cent, cyanosis was always clinically evident. Granulocytopenia also occurred but was less common. No hemolytic anemia was encountered. It should be emphasized, however, that only white subjects were used, and in these hemolytic anemia is known to be less common (11).

At 15 milligrams of pamaquin daily, there were practically no symptoms,

TABLE 3

Therapeutic effect

Pamaquin administered concurrently with quinine

PAMAQUIN ADMINISTRATION		RELAPSE RATIO INDIVIDUALS RE- LAPSED/INDIVID- UALS TREATED	DURATION OF SUBSEQUENT LATENT PERIOD		DURATION OF OBSERVATION OF NEGATIVE CASES
Base wt./day	Duration		Median	Range	
<i>mgm.</i>	<i>days</i>		<i>days</i>	<i>days</i>	<i>days</i>
15	14	4/5	14	7-22	104
15	28	3/5	45	12-96	298-300
45	14	3/5	51	50-61	121-146
63	14	2/5	58	54-63	182-220

while at 63 milligrams, abdominal pain, anorexia, nausea and vomiting were frequently severe and in several cases necessitated discontinuance of the medication before the 14 days were completed.

Although in individual cases the amount of hemoglobin converted to methemoglobin often bore no relation to the severity of the gastrointestinal symptoms, generally, a drug regime that produced severe methemoglobinemia also produced severe symptoms. The average per cent of total hemoglobin converted to methemoglobin for an entire group of patients, offered an objective numerical index of the amount of toxicity produced. The methemoglobin percentage is, therefore, used as an expression of the severity of the methemoglobinemia and an approximation of the subjective toxicity of various pamaquin regimes.

DISCUSSION

The studies described were primarily useful as a standard for the appraisal of the toxicity of other 8-aminoquinolines. Under the conditions specified, an ideal curative drug should have a toxicity no greater than that of 15 milligrams of pamaquin, which causes few or no symptoms and only minimal methemoglobinemia (3.0 per cent of the total hemoglobin). Mild toxicity is represented by the 31 milligram regime, with average methemoglobinemia of 4.9 per cent; moderate toxicity is represented by the regime of 45 milligrams

with 5.6 per cent methemoglobinemia, and severe toxicity by that of 63 milligrams with 12 per cent.³

Using pamaquin toxicity as a standard, 48 other regimes of pamaquin and other 8-aminoquinolines with pamaquin-like toxicity have been studied. Comparison of the symptomatology and methemoglobinemia of these regimes with those of the pamaquin-plus-quinine regimes made quantitative estimation of toxicity possible. For example, Table 4 shows the results obtained when pamaquin in doses of 31 milligrams a day was administered concurrently with each of five other antimalarial drugs. None of the regimes prevented relapse in more than one case out of five, but toxicity varied. SN-7618, SN-8617 and

TABLE 4

Therapeutic and toxic effect of antimalarial drugs administered concurrently with pamaquin for 14 days at a pamaquin dosage of 31 mgm. a day

DRUG*	DAILY DOSE	INDIVIDUALS RELAPSED/ INDIVIDUALS TREATED (Relapse ratio)	TOXICITY	
			Methemo- globinemia	Pamaquin equiva- lent (daily dose of pamaquin produc- ing comparable toxicity)
	gm.		per cent of total hemoglobin	mgm.
Quinacrine (atabrine)	0.3	4/5	12.1	63
SN-5241	2.0	3/3	8.8	45
Chloroquinine (SN-7618)	0.3	4/5	4.3	31
SN-8617	0.38	5/5	6.3†	31
SN-11,437 (metachlo- ridine)	1.0-1.7	5/5	4.9	31

* For a summary of available information on these compounds, see *A Survey of Antimalarial Drugs, 1941-1945*, edited by F. Y. Wiselogle, Edwards Brothers, Inc., Ann Arbor, 1946.

† The subjective symptoms in this group were slight.

SN-11,437 did not materially increase the toxicity. SN-5241 increased it moderately, and quinacrine, as had been previously reported (4), increased it strikingly. Thirty-one milligrams of pamaquin, given daily with quinacrine, resulted in methemoglobinemia and clinical symptomatology comparable to that of double the dose of pamaquin given with quinine, without an increase in therapeutic effect.

The concurrent administration of pamaquin and quinine under the conditions of these studies failed to cause the dramatic fall in relapse rate reported by others (1, 2, 3, 4, 8) in the treatment of naturally occurring malaria. Several factors may account for the discrepancy between these results and those reported by others: (1) Many patients in the field studies may have been treated during late relapses, when acquired immunity would aid the drug. (2) In others

³ The mean methemoglobin concentration of 187 normal individuals was 1.8 per cent of the total hemoglobin (standard deviation: 1.0).

natural immunity may have been higher. (3) The intensity of infection has varied considerably under field conditions. (4) Prolonged atabrine suppression may have altered the clinical course of the disease. (5) Strains of parasites have not been uniform. Differences in the results reported by various observers emphasize the need for studies under conditions where these variables are controlled.

SUMMARY

Several regimes of pamaquin (plasmochin) were given a therapeutic trial in mosquito-induced vivax malaria (Chesson strain).

The administration of daily doses of 63 milligrams of the base or 140 milligrams of the naphthoate salt, or less, concurrently with quinine for 14 days protected only a few of the subjects against relapse. No protection was afforded when the drugs were given serially. The effect was not appreciably enhanced when pamaquin (31 milligrams of the base a day) was administered concurrently with several other experimental drugs.

The average percentage of total hemoglobin converted to methemoglobin was a useful measure for the comparison of the toxicity of various regimes of pamaquin.

Standardized clinical trials diminished the influence of variables, such as acquired and natural immunity, variations in intensity of infection, the effect of suppressive drugs, and strain differences, which confuse the interpretation of results in field studies.

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* The studies reported in this paper would not have been possible except for the enthusiastic cooperation of the inmate volunteers and administrative officials of Stateville Penitentiary.

THE DETERMINATION OF THE FOLIC ACID CONTENT OF FOODS USUALLY CONSUMED BY PATIENTS WITH TROPICAL SPRUE¹

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After many decades of study, sprue, pernicious anemia, and the other related anemias have come to be accepted as being closely related by distinct clinical syndromes. They have in common a macrocytic anemia. The occurrence of Addisonian pernicious anemia is more frequent in the temperate zones while the incidence of sprue is higher in the tropics. In any large clinic one sees patients who have clearcut clinical syndromes on which all observers agree as to diagnosis. There are always a few cases, however, about which observers have differences of opinion or are unable to make up their minds. Such cases we classify as indeterminate.

In classifying the macrocytic anemias it is important to do repeated gastric analyses. In Addisonian pernicious anemia there is no free hydrochloric acid even after histamine stimulation. In other types of macrocytic anemia free hydrochloric acid is usually present. Sprue is characterized by acid steatorrhea. Observations on the bone marrow and on the peripheral blood of persons with Addisonian pernicious anemia, nutritional macrocytic anemia, the macrocytic anemia of tropical or nontropical sprue, of pellagra, and of pregnancy, reveal no distinguishing features.

After treating successfully Addisonian pernicious anemia and nutritional macrocytic anemia in relapse with synthetic folic acid, (1) we made plans to study its effect on persons with tropical sprue in Cuba and in Puerto Rico, countries in which the disease is rather common. We began our studies by making a thorough clinical and laboratory appraisal. In the selection of patients we insisted on the eight criteria shown in Figure 1.

We soon found that folic acid produced a remission in tropical sprue in a manner similar to that produced by a potent liver extract. The anemia disappeared, the patient gained remarkably in weight and in strength and, in most instances, the alimentary tract disorders became much less pronounced or disappeared entirely (2, 3, 4, 5).

The etiology of sprue has been studied by many physicians in various parts of the world. A large group came to the conclusion that sprue was primarily

¹ University of Cincinnati Studies in Nutrition at the Calixto Garcia Hospital, Havana, Cuba.

This study was aided by grants from the Instituto Universitario de Investigaciones Científicas of the Havana University and from the Martha Leland Sherwin Memorial Fund and Lederle Laboratories, Inc.

The folic acid used as a standard was supplied by Dr. E. L. R. Stokstad and Dr. Thomas H. Jukes of Lederle Laboratories, Inc. Several vitamins and other chemicals were supplied by Dr. Randolph Major of Merck and Company.

The authors wish to thank Dr. Clemente Inclan, President of the Havana University for facilities given to this work.

caused by an infectious agent. Others, especially in recent years, have thought that sprue was the result of a faulty diet. From the beginning of our studies on sprue we were impressed by the poor quality of the diet customarily eaten by our patients. It consisted chiefly of bread, cornmeal, rice viandas (starchy foods such as cassava, taro, sweet potato, ñame, and plantain), coffee, and sugar. Milk, meat, eggs, green vegetables, and fruits were included very infrequently and in small amounts, if at all. The patients' diets studied in Cuba were found to be similar to those of the cases previously reported by Milanes in Cuba (6) and by Castle, Rhoads, Lawson, and Payne in Puerto Rico (7).

When the patients were admitted to the special hospital ward for study, they were restricted to a controlled diet consisting of only those foods which were commonly consumed at home. On this diet and without therapy, no blood regeneration occurred. Soon after folic acid therapy was initiated, they improved remarkably and continued to improve despite the fact that their diets

CRITERIA FOR SELECTION OF PATIENTS

1. Macrocytic hyperchromic anemia.
2. Red blood count of 2.5 m. or less.
3. Color Index of 1.0 or more.
4. Megaloblastic arrest of sternal bone marrow.
5. Flat oral glucose tolerance test.
6. Free hydrochloric acid in gastric secretions after histamine stimulation.
7. "Fatty stools".
8. Weight loss.

FIGURE 1

remained unchanged throughout the course of the study. Since it was apparent that folic acid alone produced this effect, we were led to believe that the foods which composed the major part of the sprue patients' diet might be deficient in folic acid. Accordingly we decided to assay these foods for their folic acid content. The present communication is concerned with our findings in this regard.

MATERIALS AND METHODS

The foods were purchased at the curb markets in which our patients bought their food supplies and from which the foods included in the diets of our patients while they were in the hospital were purchased. Only the raw edible portion of these foods was assayed.

The microbiological method of Teply and Elvehjem (8) in which *Lactobacillus casei* is employed as the test organism was used. The samples were first digested with Taka-diastase as recommended by Cheldelin *et al.* (9) and then with chicken pancreas enzyme according to the recommendation of Burkholder and associates (10). Both the enzymatic hydrolysis and the microbiological assay proper were performed with minor modifications of the original methods suggested by Dr. E. L. R. Stokstad.

Briefly, the enzymatic hydrolysis procedure was as follows: The samples were

suspended in acetate buffer pH 4.5, homogenized in a Waring blender, and autoclaved at 15 pounds pressure for 15 minutes. Forty mg. of Taka-diestase were added to each sample when cool, and the resultant suspensions were incubated for 20 hours at 37°C. At the end of this period they were steamed for 10 minutes and after cooling, the pH was adjusted to 7.0. Final incubation of the extract was made with 20 mg. of chicken pancreas enzyme for 20 hours at 37°C. on a mechanical shaker. To provide more uniform temperatures for the cultures, a forced circulation incubator was employed. The readings were made by titrating with 0.1 N sodium hydroxide solution with Accutinic pH paper, which shows changes of less than 0.3 pH, being used as the indicator. Lederle's synthetic folic acid was used as a standard. The addition of Norit treated peptone to the culture medium as recommended by Teply and Elvehjem (8) proved to be useful, inasmuch as duplicate culture tubes checked much better than in a previous series of assays performed without peptone.

Digestion with Taka-diestase was particularly important since most of the foods assayed had a high starch content. During the enzymatic hydrolysis, the food extracts were handled in Kimble amber or Pyrex Low Actinic glassware. Control of the speed of the Waring blender was found necessary in many instances. Since the materials assayed were believed to contain very little, if any, folic acid, concentrated suspensions of the samples were prepared after homogenizing.

Emphasis is placed on the fact that the samples were hydrolyzed prior to assay with Taka-diestase and chicken pancreas which have been shown to split combined folic acid, thus rendering it available for the growth of the test microorganism. This method has been found to give higher and more correct values. These assays were performed with *Lactobacillus casei*, a microorganism known to be several times more sensitive to folic acid than is *Streptococcus lactis* R., another microorganism sometimes used for making folic acid determinations.

In six of the twelve samples no measurable amount of folic acid could be determined, even when as much as 250 mg. of the sample was assayed at a time. In the remaining six samples 200, 165, 165, 165, 100, and 100 mg. were the largest amounts of sample added to the tubes. These larger amounts produced no more detectable acid production than did the much smaller amounts.

RESULTS AND COMMENTS

The folic acid content of the foods assayed is shown in Table 1.

According to these assays, yellow cornmeal and rice which are two of the most commonly consumed foods in the diet of Cuban peasants (among whom most of the cases of sprue which we studied were found), contain no folic acid. No folic acid was found in coffee which is the beverage most commonly used by these persons. Of the foods assayed, yellow sweet potato showed the highest folic acid values but these values are not significant when compared to those of green vegetables assayed by other investigators (9). As can be seen from Table 1, the other foods assayed contained very little or no folic acid.

From the folic acid content of the foods shown in Table 1, foods which compose the bulk of the diet of our series of Cuban patients with sprue, it can be seen that the diets of these patients have a remarkably low folic acid content. Although it has not been proved that sprue is caused by a specific nutritional factor and although our evaluations of these diets indicate that they are deficient in several essential nutrients, we have shown that by administering only folic acid to persons with sprue, they improved spectacularly. This improvement occurred despite the fact that the patients continued to subsist on a diet composed of foods which have a low folic acid content, as shown by the assays reported in this study. These findings give added support to the hypothesis that tropical sprue is a nutritional deficiency disease.

TABLE 1

COMMON NAME	BOTANICAL NAME	FOLIC ACID (MICROGRAMS PER GRAM OF MATERIAL)
cassava.....	Manihot esculenta, Crantz	0.0004
coffee, toasted grain.....	Coffea arabica, Lin.	0
corn meal, yellow corn.....	Zea mays, Lin.	0
corn meal, yellow corn.....	Zea mays, Lin.	0
corn meal, yellow corn.....	Zea mays, Lin.	0
mango, manga amarilla variety..	Mangifera indica, Lin.	0
ñame, white.....	Dioscorea alata, Lin.	0
plantain, unripe.....	Musa paradisiaca, Lin.	0
plantain, unripe.....	Musa paradisiaca, Lin.	0
plantain, half ripened.....	Musa paradisiaca, Lin.	0
plantain, half ripened.....	Musa paradisiaca, Lin.	0.0014
rice, husked.....	Oriza sativa, Lin.	0
sweet potato, yellow.....	Ipomoea batata, Lin.	0
sweet potato, yellow.....	Ipomoea batata, Lin.	0.0016
sweet potato, yellow.....	Ipomoea batata, Lin.	0.0039
taro, white.....	Xanthosoma sagittifolium, Schott	0

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ISOSPORA HOMINIS INFECTION IN MAN

REPORT OF A CASE

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Two recent articles (1, 2) have called attention to the finding of the protozoan parasite, *Isospora hominis* in man. Since the reported number of cases, about 240, is still comparatively small it seems worthwhile to report the finding of this parasite in a returned serviceman. In previously reported cases there was little opportunity to make extended clinical observations of the patients due to their being transferred elsewhere, or to the short appearance of the organism in the stool. In this instance the parasite was observed in repeated stool specimens for several months and any clinical findings referable to the parasite could have been noted.

In the previously mentioned articles the protozoan was described and the case reports have added credence to the parasite being a pathogen in some instances. Although our patient was observed for several months no past or current history of illness referable to gastro-intestinal infection could be elicited. Physical examination and continued observation failed to reveal evidence of disease due to the parasite.

REPORT OF CASE

A soldier aged 36 was admitted to Bruns General Hospital August 3, 1945 for minimal to moderately advanced pulmonary tuberculosis. He appeared well nourished, well developed, and in outward good health. His army service was of nearly three years duration, twenty-two months of which were in tropical service. He had spent approximately five months in Australia, seven months in the Admiralties, two months in New Guinea and nine months in the Philippines. During his service on the Admiralties living conditions were very poor and he spent two and a half months in combat during which time he lived in fox holes and subsisted on K rations. During his service on the Philippines, from October 1944 to June 1945 he was in combat, sleeping in the open and subsisting on K and C rations.

On June 7, 1945 he was admitted to the 54th Evacuation Hospital on Luzon for cough and pain in chest. On June 10 an X-ray revealed a chest lesion and from that date the patient has been continuously hospitalized. Upon his admittance to Bruns General Hospital the only symptomatology given was attributable to his pulmonary condition. He gave no past or present history of diarrhea, flatulence or abdominal discomfort. Upon admittance his sputum was negative, Kahn negative, urine negative. His blood count showed 14 grams of hemoglobin, red blood count 4,610,000, white blood count 6,400, polymorphonuclears 40 per cent, lymphocytes 56 per cent, mononuclears 3 per

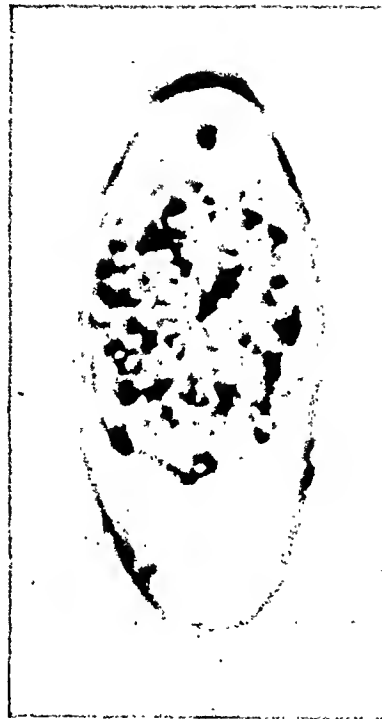


FIG. 1.



FIG. 2.

FIG. 1. ZYGOTE WITH SPOROCYST
FIG. 2. SPOROCYST IN PROCESS OF DIVISION



FIG. 3. OOCYST WITH TWO SPOROBLASTS

cent and eosinophils 1 per cent. The sedimentation rate was 32 mm/hr. Two other blood counts showed no eosinophils.

— On the fifth of September a stool was submitted for routine examination and cysts of *Endamoeba coli*, ova of *Hookworm* and *Trichuris trichiura* and a few oocysts of *Isospora hominis* were found. Between the fifth of September and November 8, 1945 repeated stool examinations were made and oocysts were consistently present. Their number varied considerably from few in number to as many as 200 oocysts per low power field by the Faust zinc sulphate con-



FIG. 4.

FIG. 4. OOCYSTS WITH DEVELOPING SPOROZOITES



FIG. 5.

FIG. 5. OOCYST WITH DEVELOPING SPOROZOITES

Forms seen in figures 1, 2, and 3, were observed in freshly passed stool. Forms in figures 4 and 5 were observed after allowing specimen to stand at room temperature for from 24 to 48 hours.

centration method. On November 16, 1945 a stool was examined and no oocysts were noted and repeated subsequent examination failed to disclose them. The patient was not treated for the presence of *Isospora* but for hookworm. If one can assume that his infection was prior to his hospitalization for tuberculosis this would point to an infection of at least six months duration. In the middle of February the patient was discharged from the hospital and returned to civilian status.

The literature has described the stools of *Isospora* infected individuals as usually being light in color, fatty and with an excess of undigested material. All stool specimens in which *Isospora* were found answered this description.

Charcot-Leyden crystals were usually present, but pus, blood and mucus were absent.

In a fresh specimen the oocysts, which measured approximately 30 micron in length by 15 micron in width, were usually undivided, the zygote containing granular protoplasm or else aggregated into a sporocyst (fig. 1). There were however, usually a few divided forms present, the sporocyst having divided to form two sporoblasts (fig. 2 and 3). If the specimen was allowed to stand at room temperature for 24 to 48 hours the developing sporozoites could be observed (fig. 4 and 5). By placing the specimen in 10 per cent formalin for one week and then transferring to 5 per cent formalin the oocysts have remained undistorted to this date, (May 6, 1946).

SUMMARY

A case of *Isospora hominis* infection in a returned soldier is described. The protozoan was observed in large numbers in the stool specimens over a period of several months. The organism spontaneously disappeared. At no time were there any signs or symptoms referable to the presence of *Isospora*. In this instance the presence of *Isospora* was concluded to be sub-clinical. The date of the onset of the infection is undetermined.

Appreciation is expressed to Dr. H. W. Brown, School of Public Health, Columbia University for confirming the opinion of the author as to the identity of the parasite.

Acknowledgment is also made to Sgt. George Chyka of the Photographic Laboratory of Bruns General Hospital for the accompanying photographs of *Isospora hominis*.

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TREATMENT OF TRICHURIASIS WITH 'ENSEALS' OF EMETINE HYDROCHLORIDE¹

ROBERT B. BURROWS², WILLIAM G. MOREHOUSE³ AND JOE E. FREED⁴

INTRODUCTION

Although *Trichuriasis* (whipworm infestation) is not important in the majority of places, it is a problem in the Southern United States in certain rural and urban areas where sanitary disposal of feces is not strictly enforced and in mental institutions (Caldwell, Caldwell and Davis, 1930; Otto, Cort and Keller, 1931; Otto, 1932; Reardon, 1941; Young and Ham, 1941; and Burrows, 1943). In this country the treatment of this infestation has been difficult, for it does not respond readily to the available drugs. Hexylresorcinol, tetrachlorethylene and oil of chenopodium will remove some of the worms (Lamson, Brown, Robbins and Ward, 1931; and Brown, 1934), but repeated treatments are necessary in most cases.

The most efficient drug is *leche de higueron*, or its active principle, ficin. *Leche de higueron* is most efficient in its unpreserved state, less so when fermented or preserved, and a solution of the freshly opened crystoid was found superior to the amorphous ficin, provided it was used immediately (Thomen, 1939; and Faust and Thomen, 1941). An extensive study was made on the toxicity of large doses of ficin, especially its effects on the intestinal mucosa and other organs (Molitor, Mushett and Kuna, 1941), but because of its proteolytic properties it was found to be safer in a series of small doses rather than in a single large dose.

Emetine hydrochloride has been put up in "enteric-sealed" tablets,⁴ the coating of which prevents the drug from being released until the tablet reaches the distal portion of the small intestine. This drug has been used in the treatment of intestinal amebiasis (Shrapnel, Johnson and Sandground, 1946). They found that no toxic symptoms resulted when the coating remained unbroken until reaching the distal part of the small intestine, but that nausea and vomiting occurred when the drug was released in the upper portion of the tract; that mild

¹ The writers wish to express their appreciation to Eli Lilly and Company, of Indianapolis, for supplying the drug for this work; to Dr. J. H. Sandground, Chief, Dept. of Parasitology, The Lilly Research Laboratories, for suggesting the use of this drug against whipworm infestations; to Miss Myrtle Rucker, R.N., for the excellent work she and her assistants did in treating the patients and collecting the specimens; and to the following laboratory assistants for their aid: Miss Kathlyn Dobson, Miss Hazel Wolfe and Mr. Walser McLendon.

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⁴ Emetine hydrochloride "Enseals" (Enteric-sealed Tablets, Lilly), each tablet containing $\frac{1}{2}$ grain of the alkaloid.

diarrhea appeared in some cases; and that toxic symptoms generally associated with parenteral administration of the drug were absent.

During the above treatment program there were indications that emetine hydrochloride "enseals" affected *Trichuris trichiura*, for eight of the 20 patients harbored this species. Dr. J. H. Sandground, in a personal communication, suggested that further investigation of this phase would be desirable. The present study was undertaken with the object of testing the efficiency of this drug against *Trichuris* and of gathering further data on the drug.

PROCEDURE

Patients Used. The program of treatment with this drug was conducted on a group of white female patients, who were confined in a building for disturbed patients. The majority were deteriorated mentally, untidy in dress and habits and, in many cases, were resistive to treatment. All had been hospitalized for periods of from four to 28 years.

Three to six pre-treatment stool examinations were made of each of the 23 patients selected for treatment and the following incidences were found:

SPECIES	PER CENT
<i>Endamoeba histolytica</i>	30.4
<i>Endamoeba coli</i>	100.0
<i>Iodamoeba williamsi</i>	30.4
<i>Endolimax nana</i>	82.6
<i>Chilomastix mcconnili</i>	78.3
<i>Trichomonas hominis</i>	56.5
<i>Giardia lamblia</i>	4.3
<i>Ascaris lumbricoides</i>	8.7
<i>Necator americanus</i>	91.3
<i>Strongyloides stercoralis</i>	52.2
<i>Enterobius vermicularis</i>	17.4
<i>Trichuris trichiura</i>	100.0

Anal swabs were not made on these patients and the above incidence for *Enterobius* was determined from the presence of adult worms found in the stools during treatment. As stools of only 13 of the 23 were examined for dead worms during treatment, it is believed that the *Enterobius* incidence was actually higher than shown.

Each patient harbored from three to ten different species of parasites, the average being 6.52 species per patient.

A study of the untidy habits of these patients explains to a large extent the reason such high incidences were found. The actually observed untidy habits of the 23 patients were as follows:

HABIT	PER CENT
Rarely wash, unless forcibly bathed.....	100.0
Go barefooted in the yard.....	91.3
Frequently put fingers in mouth.....	30.4
Geophilic, sit in or play with soil.....	47.8
Geophagic.....	13.0
Pantophagic (scraps, leaves, cloth, acorns, Hexapoda, paper, grass, etc.).....	30.4
Coprophilic.....	8.7
Coprophagic.....	8.7
Relieve selves at places other than toilets.....	8.7

Geophagicity may actually be considered as 100 per cent in this group, for it is rare for these patients to wash their hands before eating, thus transferring dirt from their hands to the food. Of the two patients not listed as going barefooted in the yard, one wore shoes and the other was not allowed in the yard due to her *mental condition*. Inasmuch as the members of this group are in a building with over 100 patients having similar habits, it is not difficult to understand the high incidences of infection found.

These 23 patients furnished a total of 150 infections of 12 different species of parasites, against which the effects of this drug could be tested.

Methods. Before these patients were treated a series of examinations were made. Each patient had the following pre-treatment examinations: (1) three to six stool examinations for species harbored; (2) three to six ova and larvae counts; (3) a differential blood count; (4) a hemoglobin determination; (5) weight; (6) blood pressure; (7) a physical examination; and (8) a mental status determination. Patients were kept in the building from the time they were selected for treatment until the post-treatment examinations were terminated, in order to minimize the appearance of new infections. That some patients did acquire new infections shortly before the pre-treatment examinations were made became evident in later examinations, for the young worms which were too far forward in the intestinal tract to be reached by the drug during treatment migrated distad, matured and their eggs were recovered during post-treatment examinations.

Where a marked difference was found either in number of species harbored or in egg counts in the first three stool examinations of a patient one to three additional specimens were obtained in order to get a more accurate picture. Egg and larvae counts were made of each specimen and the correction values recommended by Brown and Otto (1941) and others were used. Each specimen was counted two to three times and the average obtained. In order to reduce errors to a common factor all counts were made by the same person. As far as possible stools were collected after normal bowel movement, but in the cases of those chronically constipated the stools were collected after saline purgation and the most formed portions used in egg count determinations.

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Following treatment stools were collected at intervals and examined as in the pre-treatment specimens. In this way it was possible to determine decreases in number of species as well as in intensities of worm infestations. Those patients in the earlier groups who were followed for six weeks or more after treatment also had the following examinations repeated: hemoglobin determination, differential blood count, weight, blood pressure, physical examination and mental status determination.

The patients were divided into six groups, in each group the amount of drug and the number of days administered being varied. The groups were as follows: Group 1—three tablets (1 grain) daily for 12 days (5 patients); Group 2—six tablets (2 grains) daily for six days (5 patients); Group 3—nine tablets (3 grains) daily for four days (5 patients); Group 4—nine tablets (3 grains) daily for two days (4 patients); Group 5—nine tablets (3 grains) for one day (3 patients); and Group 6—16 tablets ($5\frac{1}{2}$ grains) in 24 hours (1 patient).

The first two groups of patients were treated primarily to test the effectiveness of the drug against *Trichuris* and other infections. When it was found that post-treatment examinations showed a decrease in or loss of *Trichuris* and other infections, the later groups were checked more closely. From the time treatment was begun (in Groups 3 through 6) every stool specimen passed was saved in its entirety, put into one or more containers, marked with the patient's name and time passed, and sent to the laboratory. There the specimens were washed in a fine mesh sieve and all worms removed and counted. The collection and examination of these specimens were continued for a day or two after the patient ceased to pass worms.

RESULTS

Effects of the Drug on Patients during Treatment. The 15 patients in the first three groups were given tablets after meals and the remainder (eight patients) before meals. No real differences were noted in the effects of the drug from this standpoint. There were some somatic complaints, such as nausea and vomiting, present in several patients prior to treatment. These symptoms were neither aggravated nor decreased by the drug. One patient (A. L. A.) chewed her tablets rather than swallowing them intact; yet no nausea occurred in her case. On the whole these patients did not complain of any symptoms, except nausea and diarrhea, which could be attributed to the drug itself. This may be due to the fact that these patients were mentally deteriorated, preoccupied with delusions and hallucinations and, in many instances, had poor contact with reality. Inasmuch as these tablets are recommended for pre-meal administration, such probably is preferable for the average person.

The drug had a definite laxative effect on the patients and this was more pronounced with the larger dosages. Those patients receiving three tablets three times a day generally began having loose stools from 18 to 24 hours after receiving the first tablets. A few started earlier and a few later. The stools were quite liquid, consisting mainly of fecal matter suspended in water.

During treatment and for several days afterward there was a great loss of fluid by the patients, for they had generally from four to ten movements daily and passed up to six or seven pints of fecal matter and water in a 24-hour period. Those receiving smaller dosages had fewer movements and a lower water loss.

Blood, mucus and mucosa began to appear within 24 to 30 hours after initiation of treatment, particularly in the stools of those receiving the larger dosages. Some patients showed comparatively little and others had large quantities. Occasionally pieces of mucosa of a square inch or larger were sloughed off in single pieces, though most were smaller. Many pieces of mucosa had *Trichuris* worms tangled in them. In the majority of cases blood, mucus and mucosa disappeared from the stools about the time the stools began to be formed again. One patient continued to show a little even as late as ten days after treatment was terminated. The lesions appeared to heal without any secondary infection occurring.

Effects of the Drug on Trichuris. The first two groups of patients were treated with the dosages used by Shrapnel, Johnson and Sandground (1946) for *Endamoeba histolytica*, primarily to determine the effectiveness of the drug against *Trichuris*. No check was made during treatment to determine the number of worms passed. Inasmuch as these patients showed a definite loss of *Trichuris*, it was decided to collect all stools passed during and for several days after treatment and to count the worms passed.

Of those patients receiving three tablets daily for 12 days, two lost all adult *Trichuris* from the distal portion of the intestine and the other three showed reductions in egg counts (Table 1). One patient (R. W.) still was free of *Trichuris* after six weeks, but had not been allowed in the yard for some time prior to treatment and had, therefore, acquired no new infestations.

Those receiving six tablets daily for six days showed a more pronounced loss of *Trichuris*, four of the five having negative egg counts for one to three weeks after treatment and decidedly reduced counts thereafter. At the end of six weeks the total egg count of these five patients was only three percent of the total pre-treatment count and most of this could be attributed to worms which matured after treatment, they probably being too far forward in the intestine in their younger stages to be touched by the drug.

Nine tablets a day for four days enabled three of the patients to show no *Trichuris* ova in their stools for four weeks after treatment, before some worms matured enough to produce eggs. A fourth patient (B. N.) lost over 98 percent of the adult *Trichuris* harbored in the region reached by the drug. The fifth patient (A. L. A.) refused to swallow her tablets without chewing, thus causing the drug to lose most of its effectiveness before reaching the worms. She passed one *Trichuris* during treatment and an *Ascaris* several days later. There appears to have been a further and gradual loss of *Trichuris*, for she showed reduced egg counts after treatment.

Those patients receiving nine tablets a day for one or two days failed to show as complete a loss of *Trichuris* as did those in the second and third groups. This dosage resulted in the loss of 80 to 100 percent of the adult *Trichuris*, except in

TABLE 1
Changes in Trichuris Egg Counts Following Treatment

PATIENT	PRE-TREATMENT EGG COUNT AVERAGE (FOR MED STOOL BASIS)	NUMBER TRICHURIS PASSED	POST-TREATMENT EGG COUNTS (FORMED STOOL BASIS) (No. eggs per gram of stool)						
Group 1: 3 tablets (1 grain) daily for 12 days									
No. days after treatment.....			1-2	3-6	10-11	16-20	24-30	34-35	41-43
E. B.	10,970	No counts made	0	0	0	0	0	60	350
O. C.	16,200		8,600	5,850	11,325	13,450	12,350	*	7,250
M. L. D.	17,025		3,600	75	650	810	750	*	550
L. M. G.	44,350		*	14,600	30,600	25,600	9,400	28,600	14,600
R. W.	4,030		0	0	0	0	0	0	0
Group 2: 6 tablets (2 grains) daily for six days									
No. days after treatment.....			1	3-5	10-12	16-17	23-26	29-31	40-42
S. O.	5,050	No counts made	0	0	0	0	100	500	450
V. P.	28,700		0	0	0	0	0	50	250
M. R.	25,300		0	0	0	0	150	150	225
G. S.	14,600		0	0	25	1,200	750	525	1,350
J. W.	16,530		1,000	75	300	75	300	350	550
Group 3: 9 tablets (3 grains) daily for four days									
No. days after treatment.....			5	11-14	19-20	28	36	46-49	
A. L. A.	69,740	?	36,700	15,400	20,100	38,600	*	13,600	
A. M. G.	8,030	54	0	0	0	0	0	375	
B. N.	55,600	562	900	400	40	960	1,200	5,550	
E. P.	16,900	466	0	0	0	0	50	0	
W. T.	108,700	2,538	0	0	0	0	0	225	
Group 4: 9 tablets (3 grains) daily for two days									
No. days after treatment.....			7-9	9-11	20	30	38		
H. M. B.	19,740	71	*	2,500	1,440	2,025	4,500		
B. C.	16,315	168	2,250	2,850					
R. M. F.	99,360	639	5,000	6,375					
M. C.	23,070	27	0	0					
Group 5: 9 tablets (3 grains) for one day									
No. days after treatment.....			7-8	9-12					
M. B.	1,250	0	1,100	875					
M. I.	22,450	54	2,375	3,050					
D. K.	20,075	306	1,100	2,175					
Group 6: 16 tablets (5½ grains) in 24 hours									
No. days after treatment.....			14	21	32	39			
S. K. C.	1,500	14	0	0	75	60			

* Indicates that no egg count was made at that time.

one patient (M. B.). Not a worm was recovered from her stools and her post-treatment egg counts were nearly as high as the pre-treatment average.

One patient (S. K. C.) was given 16 tablets in 24 hours to determine tolerance of the drug. This amount apparently removed all adult *Trichuris* in the distal part of her tract, for her stools were negative for over three weeks.

The Rate of Elimination of Trichuris. Those patients receiving nine or more tablets daily had all stools collected for five to six days after treatment was begun.

TABLE 2

Rate of Loss of Trichuris after Treatment

(Figures Represent the Cumulative Percentages of Total Recovered Worms)

PATIENT	NUMBER TRI- CHURIS PASSED	HOURS AFTER TREATMENT WAS BEGUN										
		24	30	36	48	60	72	84	96	108	120	132
Group 3: 9 tablets (3 grains) daily for four days												
A. L. A.	?											
A. M. G.	54			3.7	55.6	77.8	98.1	100.0				
B. N.	562		.5	.5	17.3	82.0	95.0	98.7	99.4	100.0		
E. P.	466		51.5	51.5	90.8	95.5	98.1	100.0				
W. T.	2,538			5.1	21.8	42.6	55.3	68.3	87.3	99.9	100.0	
Group 4: 9 tablets (3 grains) daily for two days												
H. M. B.	71		9.9	9.9	36.6	63.4	74.6	98.6	100.0			
B. C.	168		14.3	22.0	51.8	90.6	99.4	99.4	99.4	100.0		
R. M. F.	639		55.5	55.5	55.5	77.5	77.5	94.4	98.9	99.8	100.0	
M. C.	27		3.7	3.7	74.1	74.1	88.9	96.3	96.3	96.3	100.0	
Group 5: 9 tablets (3 grains) for one day												
M. B.	0											
M. I.	54				77.8	100.0						
D. K.	306	.3	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	60.5	100.0
Group 6: 16 tablets (5½ grains) in 24 hours												
S. K. C.	14		21.4	35.7	43.0	100.0						

These specimens were washed and all worms recovered. The rates at which the worms were lost are shown in Table 2.

One patient passed a worm 18.5 hours after receiving the first tablets, but the majority did not begin until 24 to 30 hours after initiation of treatment. These patients passed over 50 per cent of the recovered worms by the thirtieth hour and then lost them more slowly for the next two to four days. By the sixtieth hour all patients, with one exception, had passed from 60 to 100 per cent of the total *Trichuris* recovered.

One patient (D. K.), who normally had only two to three bowel movements weekly, passed 60 per cent of the *Trichuris* within 30 hours of the initiation of

treatment. No further stool was passed until the sixth day, at which time she was given a dose of salts. Dead *Trichuris* in the stool she then passed were embedded in hard fecal matter and could not be recovered for counting until the specimen had remained in deci-normal sodium hydroxide solution over the week-end.

There appears to be no correlation between the rate of loss of *Trichuris* or the number passed and the quantity of drug given.

Effects of the Drug on Hookworm. Twenty-one of the patients harbored hookworm and the effects of the drug on this infestation were studied. Few adult worms were found in the stools collected during and immediately after treatment. However, definite reductions in hookworm egg counts were found in post-treatment examinations of all but two of the patients (Table 3). One-third of these patients showed no hookworm ova for one to three weeks following treatment.

From these data it is apparent that the drug did eliminate some of the worms in nearly every patient and all of the worms in others. However, there does not seem to be a definite correlation between number of worms harbored, based on egg counts, and percentage lost. As in the case of *Trichuris*, egg counts increased in the weeks following treatment, as new hookworms acquired just prior to treatment became mature.

Effects of the Drug on Enterobius. Prior to treatment no anal swab examinations were made on these patients. One (H. M. B.) passed pinworm ova in one pre-treatment stool. However, during treatment adult *Enterobius* were recovered from four patients (H. M. B., B. C., R. M. F. and D. K.), who passed 226, 21, 3 and 5 worms, respectively.

Inasmuch as adult *Enterobius* are recovered occasionally from stools following saline purgation, it is not known definitely whether the worms recovered from the above patients were lost as a result of the vermicide or the laxative effect of the drug. Further research is needed, especially on children in institutions, where pinworm infestations are more prevalent.

Effects of the Drug on Other Parasites. Two patients (L. M. G. and A. L. A.) harbored *Ascaris* before treatment. The former had an average egg count of 8,375, formed stool basis, before treatment. After treatment she showed a count of 3,000, which later climbed to 6,215 by the end of the fifth week. Apparently she lost about half of her worms during or immediately after treatment. The other patient (A. L. A.) had a pre-treatment egg count average of 900. On the ninth day after treatment was initiated she passed one female *Ascaris* and thereafter her stools were negative for this parasite. One patient (O. C.) showed no *Ascaris* at all in her stools until the sixteenth day after treatment ended, at which time she had a count of 200 eggs per gram of stool. This had climbed to 4,350 by the end of the sixth week. Apparently she acquired her infestation shortly before she was treated.

Larvae counts were made at each examination of the 12 patients harboring *Strongyloides*. No patient lost all worms and in the majority no consistent reduction in numbers of larvae per gram of stool was found. Thus, this drug appears not to be effective against this parasite.

TABLE 3

Changes in Hookworm Egg Counts Following Treatment

PATIENT		PRE-TREAT- MENT EGG COUNT AVERAGE (FORMED STOOL BASIS)	POST-TREATMENT EGG COUNTS (FORMED STOOL BASIS) (No. eggs per gram of stool)						
Group 1: 3 tablets (1 grain) daily for 12 days									
No. days after treatment...			1-2	3-6	10-11	16-20	24-30	34-35	41-43
E. B.	670		100	250	50	400	750	500	650
O. C.	630		0	0	40	250	200	*	350
M. L. D.	1,360		600	75	450	3,200	2,375	*	1,850
L. M. G.	8,375		*	3,000	4,200	3,000	400	6,215	6,000
Group 2: 6 tablets (2 grains) daily for six days									
No. days after treatment...			1	3-5	10-12	16-17	23-26	29-31	40-42
S. O.	180		100	125	150	150	450	400	300
V. P.	3,000		0	0	300	450	1,300	1,350	2,700
M. R.	1,530		0	0	0	0	450	600	600
G. S.	7,150		0	0	0	1,500	5,940	8,025	5,650
J. W.	530		0	0	100	150	1,350	800	1,400
Group 3: 9 tablets (3 grains) daily for four days									
No. days after treatment...			5	11-14	19-20	28	36	46-49	
A. L. A.	900		600	400	600	1,575	*	200	
A. M. G.	1,700		*	2,190	2,400	2,050	1,200	3,000	
B. N.	370		100	100	40	50	150	450	
E. P.	215		0	0	0	0	200	0	
W. T.	1,230		0	150	100	450	375	675	
Group 4: 9 tablets (3 grains) daily for two days									
No. days after treatment...			7-9	9-11	20	30	38		
H. M. B.	460		*	650	375	225	500		
B. C.	895		975	600					
R. M. F.	3,810		2,310	2,690					
M. C.	8,335		6,150	5,100					
Group 5: 9 tablets (3 grains) for one day									
No. days after treatment...			7-8	9-11					
M. I.	1,940		250	1,100					
D. K.	1,490		300	600					
Group 6: 16 tablets (5½ grains) in 24 hours									
No. days after treatment...			14	21	32	39			
S. K. C.	5,240		875	3,450	3,450	2,690			

* Indicates that no count was made at that time.

The flagellates (*Chilomastix*, *Trichomonas* and *Giardia*) were not affected by this drug, for they continued to be found in stools immediately following treatment.

For one to three weeks following treatment the various amebae were absent or greatly reduced in numbers. Then some began to reappear. The patients in the first three groups were checked frequently over a period of six weeks following treatment and the following amebic infections failed to reappear in that time: four of the five cases of *Endamoeba histolytica*, two of the 15 cases of *Endamoeba coli*, six of the 13 cases of *Endolimax nana* and both cases of *Iodamoeba williamsi*. Inasmuch as amebae vary from day to day both in presence and in intensity, even under normal conditions, it would be necessary to make daily examinations and cultures following treatment to determine to what extent this drug is effective against the non-pathogenic amebae. From the above data it appears to be more effective against *Endolimax* and *Iodamoeba* than against *E. coli*, yet it cannot be considered specific against these amebic infections in the dosages given.

Changes in Patients Following Loss of Worms. The 15 patients comprising the first three groups were observed closely for six weeks after treatment and at the end of that period an evaluation was made of the effects of the treatment upon these patients. This paper was prepared before a similar period of time elapsed for the remaining eight patients.

The loss of *Trichuris* had no apparent effect upon the mental condition of these patients. In caring for them the nurse found that several became more cooperative and possibly a little quieter after treatment. However, it is generally known that during the course of any mental illness there are periods of improvement without any known reason or cause.

Nine of the 15 patients, or 60 percent, showed an increase in weight at the end of six weeks, the increases ranging from two to 13.5 pounds. The greatest gain was made by the patient who passed 2,538 *Trichuris*. In six of the patients the weight remained unchanged or showed a decrease. However, two of these were ill during the period of observation, one with a breast abscess and the other with acute pleurisy, which was not tubercular.

Prior to treatment these 15 patients all had definite eosinophilia, ranging from seven to 45 percent. Six weeks after treatment 12, or 80 percent, had an average decrease in eosinophil count of five percent. Of the three who showed increased eosinophilia the following information may account for them: one patient (O. C.) began to show *Ascaris* ova on the sixteenth day after treatment, although this worm had not been present in any previous examination; and two patients (L. M. G. and A. L. A.) lost only about half of their *Trichuris* and continued to harbor two to three other worm infestations.

No significant changes with respect to hemoglobin or blood pressure determinations could be observed.

DISCUSSION

In its ability to eliminate *Trichuris* infestations, emetine hydrochloride, in enteric-sealed tablets, appears to be equal to or superior to leche de higueron. The latter drug is not capable of removing all worms in all patients (various

workers quoted by Thomen, 1939), and repeated treatments are necessary in many cases. The dosages of emetine hydrochloride used in the present study removed 88 percent of all adult worms in the distal portion of the intestinal tract of the 23 patients, as shown by egg counts. And 11 of the patients apparently lost all adult worms then located in the part of the intestine in which the drug acted. Increased egg counts in later post-treatment examinations showed that some worms had matured and had migrated distally after treatment had been completed. Lack of full cooperation by several of the patients in taking the tablets may account for the fact that a higher percentage of elimination was not obtained.

Due to the fact that the latex of fig trees varies considerably with the species of tree and with the time of year collected (Thomen, 1939), leche de higueron does not possess the uniformity of action exhibited by emetine hydrochloride tablets. From this standpoint emetine hydrochloride may be considered superior as an anthelmintic.

Emetine hydrochloride appears to be less drastic in its action than ficin, the active principle of *Ficus latex*. Ficin shows some digestive action on the worms, causing digestion of portions or a disintegration of entire worms (Faust and Thomen, 1941). In the present study the worms were found to be killed by the action of emetine hydrochloride, but to be free of digestion or fragmentation.

In its action on the patient emetine hydrochloride appears to have some of the features of leche de higueron and ficin. Some nausea and vomiting occurred, although these were not as prevalent as diarrhea, which was present to some extent in all patients. There was some sloughing of intestinal mucosa, as well as some blood in the stools of some patients. This occurred mainly when diarrhea was at its height and worms were often found coiled in the mucosa. There is a question as to whether the drug caused the sloughing or the struggles of the dying *Trichuris* tore away the mucosa. In the majority of cases the presence of blood, mucus and mucosa in the stools ceased about the time diarrhea was terminated and formed stools were again passed. Ficin was found to cause erosion of the intestine, disintegration of the stomach and intestine and partial digestion of certain other organs when given in large doses, but these undesirable effects could be mitigated by giving smaller dosages (Molitor, Mushett and Kuna, 1941).

Emetine hydrochloride does not appear to be as efficient as tetrachlorethylene or hexylresorcinol for the removal of hookworm or *Ascaris*, but does cause some loss of these worms. Some *Enterobius* were passed during treatment. However, further research will have to be carried out to determine this drug's efficiency against *Enterobius*.

From the present study it appears to be evident that emetine hydrochloride, in enteric-coated tablets, will be the drug of choice in the treatment of *Trichuris* infestations. The optimum dosages will have to be worked out, preferably on non-psychotic adult patients and on children, before it can be used extensively.

SUMMARY AND CONCLUSIONS

1. Emetine hydrochloride, in enteric-sealed tablets, was used to treat *Trichuris* infestations in 23 patients. Approximately 88 percent of the adult worms were lost by this group, 11 losing all adult worms then in the distal part of the intestine.

2. This drug caused some elimination of *Enterobius*, *Ascaris* and *Necator*. However, it did not appear to be as effective against *Ascaris* and hookworm as are other drugs.

3. Nausea and vomiting occurred in a few patients and diarrhea was present to some extent in all patients. Blood, mucus and intestinal mucosa appeared in the stools of most of the patients during diarrhea, but disappeared as a rule when stools became formed again. No secondary infections developed and no ill effects were found, other than the above.

4. Six weeks after treatment the majority of patients showed an increase in weight and a decrease in eosinophilia as a result of loss of most of the *Trichuris*, even though other infestations were still present.

5. Emetine hydrochloride, in enteric-sealed tablets, appears to be less variable in its action than leche de higueron and less drastic in its action on the patient than ficin in large dosages. Therefore, it is believed that it may be found to be the drug of choice for *Trichuris* infestations.

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PIPERONYL BUTOXIDE, A NEW AND SAFE INSECTICIDE FOR THE HOUSEHOLD AND FIELD

WALTER E. DOVE*

Among the different insecticides tested for the armed forces, the most promising for use with pyrethrum proved to be piperonyl cyclohexenone. Because of its efficiency, safety, and the fact that it was activated by small amounts of pyrethrum, extensive studies were made on the insecticidal properties of compounds that were related to it, by Schroeder, Jones and Lindquist (1). The results were so encouraging that similar studies were made of a related compound.

The present paper reports observations were made upon another insecticide of this group known as piperonyl butoxide. It was found to be a safe and stable insecticide that kills many species of insects at reasonably low concentrations. When it was combined with small amounts of pyrethrins, the combination afforded a rapid knockdown, a greater mortality, and a long residual action. The stability of the combination was strikingly shown by the insecticidal properties of residues that are left from applications of surface sprays.

PHYSICAL PROPERTIES

Piperonyl butoxide, as an industrial product, is practically odorless, is a pale yellow oily liquid, and has a specific gravity at 25°C. of about 1.06. In its pure state it is clear. It possesses a faint bitter taste that is evident several seconds after it is exposed to the tip of the tongue. This insecticide is soluble in all dilutions in mineral oils commonly used as solvents, forms clear solutions with liquified gases that are used as propellents for aerosols, and is neutral to litmus. Because its moisture content is zero for all practical purposes, there is no non-volatile residue. Piperonyl butoxide is satisfactory in stable emulsions, for impregnating dusts, and for wettable dusts that are suitable for use as sprays or dips.

METHODS

In testing household space sprays, the Official Test Insecticide of the National Association of Insecticide and Disinfectant Manufacturers (100 mg. of pyrethrins in 100 ml. of deodorized base oil) was used as a standard for comparing different dilutions of Piperonyl butoxide and pyrethrins (1). Sprays were applied in Peet-Grady chambers, against houseflies that were reared according to the specified procedure. At a dosage of 12 ml. of the O.T.I. in 216 cu. ft. chambers there was a complete knockdown of flies within 10 minutes, and a mortality of approximately 50 per cent of the flies within a period of 24 hours. With this standard of performance as a comparison, different proportions of piperonyl

* Contribution of the Entomological Development Laboratory, Dodge and Olcott, Inc., Baltimore, Md.

Read at a meeting of the American Society of Tropical Medicine, Miami Beach, Florida, November 6, 1946.

butoxide and pyrethrins were tested, and the most efficient concentrations of these insecticides were determined against houseflies. Subsequently, the same method was used against mosquitoes and confused flour beetles, but greater concentrations were tested against cockroaches.

In testing aerosols produced by liquified gases, as well as those made with concentrated sprays by hand sprayers, houseflies were used in large chambers like those employed by Schroeder and Lindquist (3). The procedure was similar to that for spray tests in Peet-Grady chambers. Because these chambers have a capacity of 3350 cu. ft., it was possible to accurately weigh the quantities of insecticides desired for a single test and to use quickly the entire sample. It was found that the results obtained in this manner were quite comparable to those obtained under conditions of average use. Since 3 grams of liquified aerosols are recommended and used for obtaining a high degree of mortality of flies in 1,000 cu. ft., comparative studies of formulas were made at dosages of 1 or 2 grams per 1,000 cu. ft. of space and with exposures of the flies for 2 or 5 minutes.

In testing surface sprays, unpainted plywood panels measuring one square foot, were sprayed with 4 or 5 ml. of spray. When 8 ml. of spray was used, the panels were too wet and it was necessary to keep them in a horizontal position for drying. After the panels dried, houseflies were exposed under Petri dishes to the treated surfaces. As soon as all of the flies were knocked down, they were transferred to clean cages, and were provided with food and water. At the end of 24 hours, the mortalities were recorded.

In determining the oral acute toxicity of piperonyl butoxide to warm blooded animals, use was made of laboratory rats, rabbits, and dogs. The safety of the material permitted dosages in milliliters per kilogram of body weight, which were administered by means of a stomach tube.

HOUSEHOLD SPACE SPRAYS

In household sprays, combinations of pyrethrins and piperonyl butoxide furnished an increased mortality of houseflies. In Peet-Grady tests made with a sample containing only 40 mg. of pyrethrins, (fig. 1) there was a mortality of 31 per cent of the houseflies that were knocked down, or 34 per cent of all flies used in the tests. In a similar manner a sample containing only 400 mg. of piperonyl butoxide gave a mortality of 12 per cent of the "knockdown" flies, or 65 per cent of the total number of flies used in the test. When 40 mg. of pyrethrins and 400 mg. of piperonyl butoxide were combined in one sample and were tested, there was a mortality of 93 per cent of the knocked down flies, or 94 per cent of all flies used in the test.

There was also a striking difference in the speed of action of the combination of these two insecticides (fig. 2). At the end of a ten minute exposure a spray containing 400 mg. of piperonyl butoxide gave a knockdown of 22 per cent of the flies, while a different one containing 40 mg. of pyrethrins gave a knockdown of 82 per cent of the flies. When 40 mg. of pyrethrins and 400 mg. of piperonyl butoxide were used together, the knockdown was 98 per cent of the flies.

By studying different combinations of these two insecticides in sprays, as indicated in the graphs (figs. 1 and 2), it was determined that the most efficient proportion was a ratio of one part of pyrethrins to eight parts of piperonyl butoxide. From these investigations a household spray was developed which affords a rapid knockdown, and a mortality of flies that is about 70 per cent greater than that of the Official Test Insecticide. Such a spray permits the use of smaller quantities for killing fleas, mosquitoes and sand flies, while increased

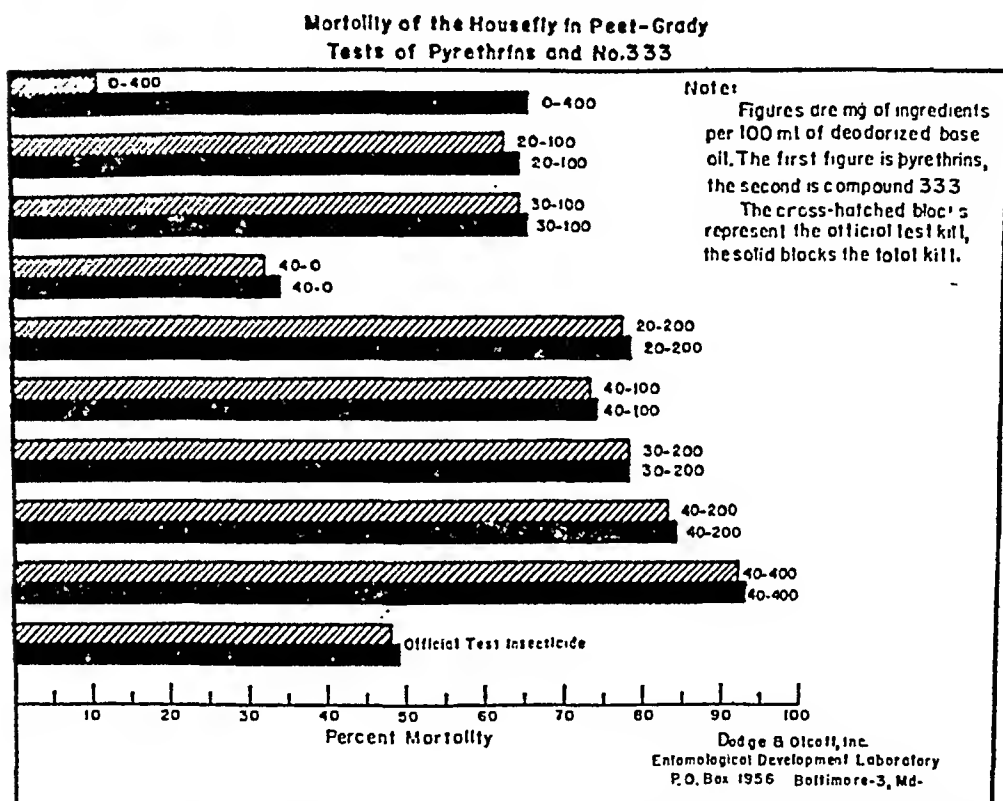


FIG. 1. Per cent mortality of house flies in Peet-Grady chambers sprayed with different proportions of pyrethrins and piperonyl butoxide in odorless base oil. Mortality of the Housefly in Peet-Grady Tests of Pyrethrins and No. 333.

amounts give good mortalities of such insects as clothes moths, bed bugs, silver fish, and confused flour beetles.

SPECIAL SPRAYS FOR COCKROACHES

Because the household spray indicated above gives a mortality of about 50 per cent of the cockroaches, larger rates of application of it were necessary for control of cockroaches. A more concentrated spray that makes use of increased amounts of pyrethrins and piperonyl butoxide in a proportion of one of the former to five of the latter, was found to be quite satisfactory for control of American, German, and Oriental cockroaches. In the laboratory and in practical tests, a concentrated spray was diluted effectively for use in conventional type sprayers,

in steam dispensers, and in electric vaporizers. The more dilute sprays were more effective when applied as large droplets by conventional type sprayers, while the more concentrated solutions gave better performance in the other devices that delivered particles of smaller sizes.

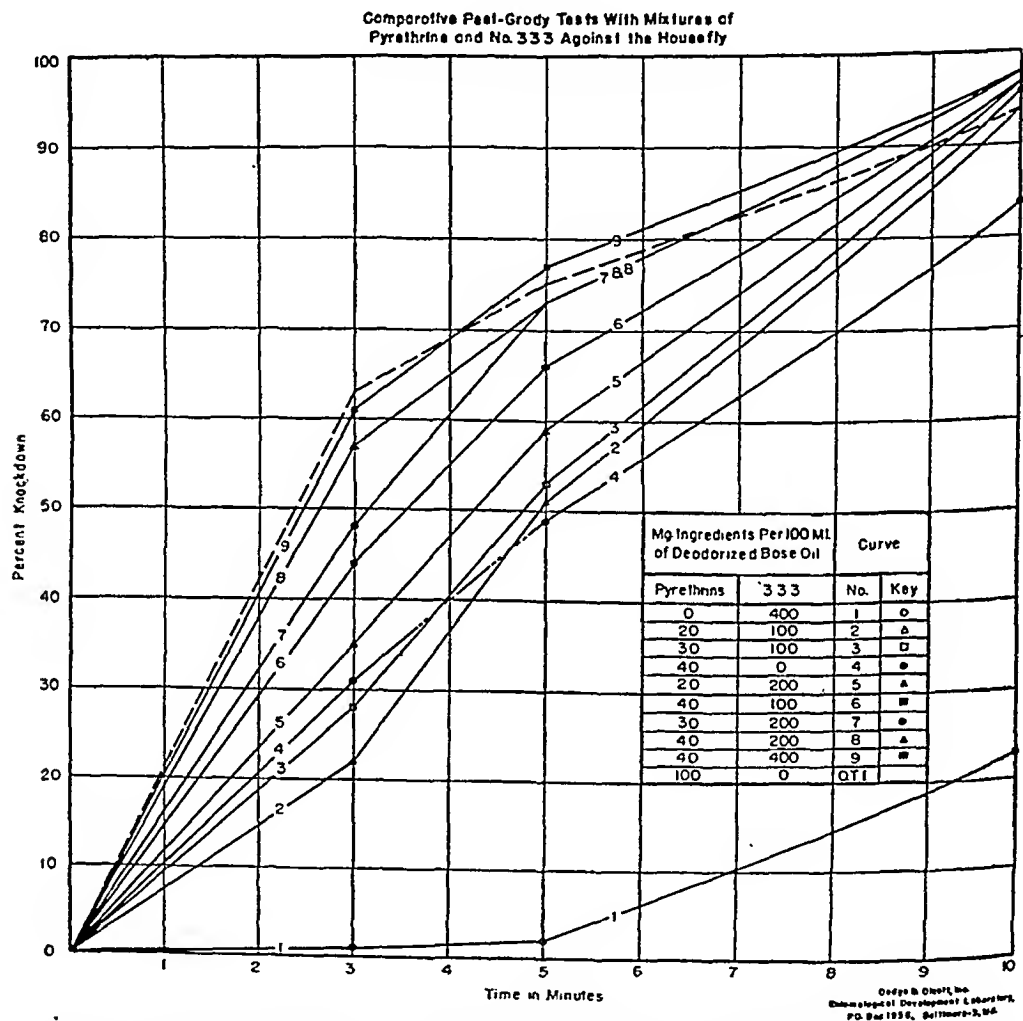


FIG. 2. Rapidity of knock down of house flies sprayed in Peet-Grady chambers with different proportions of pyrethrins and piperonyl butoxide in odorless base oil.

HOUSEHOLD SURFACE SPRAYS

When a wall is sprayed and a dry film of insecticide is left which is capable of killing or repelling insects that rest on the treated areas, the application is considered a surface or residual treatment. Until recently, this method of use was almost entirely associated with one of the chlorinated hydrocarbons. Other insecticides, due to their own stability and stabilizing influences, also possess characteristics that make them useful in surface treatments. In tests on plywood panels, piperonyl butoxide was combined with small quantities of pyreth-

rins, and was applied in deodorized base oil at various rates of application. All of the larger concentrations were effective against houseflies in the laboratory for a period of seven months, with only a slight indication at the present time of depletion of effectiveness. This indicates that piperonyl butoxide is not only a highly stable insecticide, but also that it has stabilized the pyrethrins in the surface film. At the end of 131 days, a plywood panel treated with 5 mg. of pyrethrins and 100 mg. of piperonyl butoxide in odorless base oil gave a knock-down of 100 per cent of the flies within one hour, and a complete mortality of them. Panels receiving the same amount of pyrethrins and one-half as much piperonyl butoxide were highly effective for 6 to 8 weeks, and partly effective for 5 months.

Water dispersible powders containing piperonyl butoxide and pyrethrins have shown remarkable efficacy in surface treatments of unfinished wood and other porous materials. The amounts of powder used in the films were not sufficient to mar surfaces or finishes. Apparently surface sprays of this type remain on the exterior where the insecticidal ingredients are readily contacted by insects that visit or rest on the treated areas.

Surface sprays were most efficient when they were applied to such resting places of insects, as screened doors, screened windows, portions of outside walls where flies congregate, inside of window panes of glass, on the corners of stanchions in barns and along the edges of posts or overhead beams. Treatments can be applied to selected places so that the dead flies will be restricted to certain portions of the building. It is important to select such places for treatment, so that insects exposed to the treated areas will drop in places where they cannot contaminate foods. It is advisable to treat favorable locations outside of buildings so that insects can be prevented from gaining entrance to the buildings.

DUSTS FOR THE HOUSEHOLD

Dusts containing pyrethrins 0.16 per cent and piperonyl butoxide 1.0 per cent have given very satisfactory control of cockroaches. The dusts were applied to floors near baseboards and on shelves, where the roaches could walk upon treated surfaces. These treatments gave a quick kill of the roaches, and remained effective for a period of 60 days or longer. The dusts were also found to be effective on tropical rat mites and household ants, and should be tried against Brown dog ticks in buildings.

HOUSEHOLD AEROSOLS

The conveniences and efficiency of insecticidal aerosols have had much to do with their wide and successful uses, and it is expected that piperonyl butoxide will add much to the popularity they have enjoyed. Combinations of pyrethrins and piperonyl butoxide, at a proportion of one part of the former to eight parts of the latter, by weight, provide equal knockdown and greater mortalities of insects than the formulae used in the past. Also this combination now makes it possible to eliminate certain chlorinated hydrocarbon insecticides or to greatly

reduce the quantities formerly employed. This means that auxiliary solvents required for such insecticides, may be reduced or eliminated. It means that there will be less opportunity for corrosion or clogging of the nozzles of the dispensers. It means also that the user will avoid much of the irritation and odor that are inherent in some solvents that have been used in aerosols.

When the new aerosols are used at the rate of 3 grams per 1,000 cu. ft., they are very effective against such insects as flies, mosquitoes, fleas, ants, clothes moths, sand flies, gnats, and leaf hoppers. Repeated uses tend to form residues that are capable of killing such insects. Increased dosages are applied in the regular manner for killing such insects as bed bugs and cockroaches. Such crawling insects may be killed conveniently by directing the aerosols into their hiding places.

FIELD AND GARDEN CROPS

A concentrated emulsion diluted at the rate of one part of the concentrate with 399 parts of water and sprayed on beans, potatoes, and squash, protected such crops from insect injury. The Mexican bean beetle and the Colorado potato beetle, were controlled by single treatments for periods of about 17 days. The tarnished squash bug reappeared on treated plants two weeks after they were sprayed.

Against aphids and leaf hoppers satisfactory results were obtained with both dusts and sprays on both gardens and field crops.

On alfalfa, both sprays and dusts gave a high degree of protection against a variety of insects, and there was an increased yield of hay due to its freedom from insects.

Against the blueberry maggot, a single treatment made late in the season furnished about 70 per cent control of this pest.

DOMESTIC ANIMALS

Emulsions or light oil sprays containing piperonyl butoxide and pyrethrins, were applied to dairy animals in barns just as cattle fly sprays are used by a dairyman. The treatments of 2 ounces per animal gave complete repellency to horn flies for 4 hours. Horn flies and mosquitoes were killed by direct application of the sprays, also by the residues that formed from repeated use on the hair of the animals. Greater concentrations in oil or emulsions killed stable flies that attempted to feed on treated animals. Even stronger emulsions protected livestock from the large horse flies (*Tabanus*) and deer flies (*Chrysops*) for periods of 48 hours or longer. In one field test in Kansas, with an exceptionally strong emulsion, the livestock owner reported that his animals were protected against such flies for approximately two weeks.

TOXICITY

Piperonyl butoxide was found to be safe, and free of any normal hazards of toxicity. It was well tolerated in large quantities by warm blooded animals.

In combination with pyrethrins, the concentrated solutions were found to be no more toxic to laboratory animals than the base oils used in making the solutions. The MLD 50 of the compound for rabbits appears to be about 5.0 ml./kg. The MLD 50 for rats and dogs is between 7.5 and 10.0 ml./kg. This means that a single dose necessary for an MLD 50 for man would be somewhat in excess of one pint of piperonyl butoxide.

PROBABLE USES

A wide usage is indicated for this new insecticide. Because of its efficiency and freedom from hazards of toxicity to warm blooded animals, combinations of piperonyl butoxide and pyrethrins will undoubtedly find extensive uses for safely controlling insect pests about food processing plants, in food establishments, in the household, and on crops that serve as foods for man or domesticated animals.

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SCHISTOSOMIASIS JAPONICA

A REPORT OF ITS DISCOVERY IN APPARENTLY HEALTHY INDIVIDUALS¹

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Schistosomiasis japonica, like malaria and filariasis, is a tropical disease which both military medical officers and private physicians may expect to encounter in military personnel who have returned to the United States from the Pacific Theater. Because its initial symptoms are often so mild, its presence may go undetected for many months, and, in fact, may not be discovered unless a careful search is made for diagnostic signs. This paper deals with a survey made by medical officers of a numbered station hospital in the Philippine Islands on the apparently healthy personnel operating the hospital. Since this survey was made (in March and April, 1945), it has come to the authors' attention that a similar study (1) was carried out by medical officers in Puerto Rico, where ten percent of the personnel examined were found to have a definite *Schistosoma mansoni* infection although they had never complained of any symptoms of the disease.

PRE-SURVEY FINDINGS

Before the hospital began operations at its present location (Mindoro), the organization was staged for some weeks on another of the Philippine Islands where schistosomiasis had long been known to be endemic (Leyte). Among the first patients admitted from Mindoro were many soldiers who had spent from one to several weeks staging at Leyte, and it was known that many of them had taken their daily bath in a river heavily infested with the cercariae of *S. japonicum*. A majority of these admissions entered the hospital with various, often vague complaints which suggested other tropical diseases than schistosomiasis. Some of them had episodes of fever with intervals of apparent recovery, not unlike malaria; others had symptoms which simulated an acute arthritis, allergic manifestations, or sometimes such isolated gastro-intestinal disorders as bloody stools, constipation, diarrhea, or both. Since the hospital had not been properly warned of the presence of *S. japonicum* on Leyte, the possibility of schistosomiasis as a diagnosis did not at first occur to the medical officers, and it was not until rising eosinophile counts were observed in some of the patients that more careful investigations were made in this direction. The stools of these patients were examined, and in a large percentage were found ova of *S. japonicum*. Thereafter all patients whose histories disclosed that they had staged in the endemic area on Leyte had their stools routinely checked for ova, no matter what the admitting diagnosis. As a result, a number of cases of clear-cut schistosomiasis were found among patients whose symptoms on admission had been regarded as due to other diseases.

¹ The authors are indebted to Karl L. Sicherman, Major, M.C. AUS who edited this article for publication.

In the examination of these patients, two diagnostic criteria were particularly helpful, namely, the finding of schistosome ova which were smaller than the adult eggs described in the available textbooks (2), and the lesions observed on proctoscopy. Ova were often seen which were about half the size of the usual adult egg, but otherwise similar except for incomplete development of the miracidium. Typical adult ova were of course also observed, either in the same specimen as contained the smaller ova or in later specimens from the same patients. These smaller ova were subsequently identified by an officer from an army medical laboratory (4) as immature or possibly degenerate ova of *S. japonicum*.

As interest in the problem increased, it was decided to proctoscope some of the patients who had negative stools but high eosinophilia; and lesions were observed in the rectosigmoid and sigmoid mucosa which were not recognized by the proctologist as anything he had previously seen. References to the meager literature available brought to light the strong similarity between the lesions described as typical of schistosome infection and those noted in the present series of patients (1). Several patients were promptly re-proctoscoped, and scrapings were secured from typical lesions which had been opened with a long bistoury. Indubitable *S. japonicum* ova were found in these scrapings, but this type of biopsy was discontinued when it was brought to the attention of the medical officers that the procedure may lead to complications (4). Thus diagnoses of schistosomiasis were made in some cases by finding ova in the stools; in others, by the discovery of typical lesions on proctoscopy; in others, by a concomitant discovery of ova and mucosal lesions; in a few, by biopsy of typical lesions and the recovery of ova from them; and in others, by proctoscopic findings which were later substantiated by the identification of ova in the stools.

The proctoscopic findings were of particular interest. They varied from minute, patchy areas of distended venules to similar areas with ulcerations in the sigmoid and rectosigmoid portions of the bowel. All of the lesions were rather sharply circumscribed; the surrounding mucosa was normal, and no case of generalized mucosal inflammation was found. No lesions from late stages of the disease were seen, for all of the cases observed were of not more than one to four months duration. The distended venules occurred in a characteristic stellate pattern, and when they were multiple, they were diffusely and patchily distributed, standing out sharply against the otherwise normal mucous membrane. In some of these stellae, at the bifurcation of the venules, were seen minute, grayish and yellow glistening plaques the size of a pinhead or smaller, which had the appearance of an early blister or pustule. When the "blister" was scraped, the ulcer (described below) was readily visualized (1). Faust states that these plaques or pseudo-tubercles are forerunners of the true ulcer stage.

The ulcers ranged from pinpoint to pinhead size. They were sharply defined, rather shallow, with straight, unblurred margins, and they bled very freely when they were touched. They were found for the most part at the bifurcation of venules, although a few were also found along the course of venules.

A linear ulcer 2 to 3 millimeters in length was occasionally seen paralleling a venule.

An interesting finding in two early cases was a patchy type of bronchopneumonia found only by X-ray films. Symptoms were cough, hemoptysis, and elevated temperature, and were regarded as probably due to pulmonary invasions.

The eosinophile count in about 60 per cent of the active cases varied from 20 to as high as 76 per cent. Eosinophilia, however, was not regarded as an essential early diagnostic criterion (3, 4), for in many cases there was no increase in eosinophiles even when both positive stools and positive proctoscopies were reported.

During the early weeks of the hospital's functioning at Mindoro, it did not occur to any of the staff to ponder their own recent residence in the hyper-endemic area of Leyte. This neglect was due to two main factors; first, since the hospital was overcrowded well beyond capacity there was little time for reflection; and second, a failing common among physicians, the sublime faith that the diseases they treat can never happen to them. Late in March, however, one of the enlisted ward attendants was admitted to the hospital with complaints of alternating constipation and diarrhea, myalgic pain in the neck, and a moderate loss of weight, a common group of symptoms among soldiers in the tropics, especially when they have been long fatigued and have had insufficient rest and recreation. In addition, however, this soldier's liver was just palpable and a routine blood examination revealed an eosinophilia of sixteen per cent. Two successive stool examinations were negative for ova, but the patient was proctoscoped and the typical picture of schistosome infection, distended venules and minute ulcers, was seen. Smears from the lesions were negative for ova, but subsequent stool studies were positive; and when the eosinophile count rose to twenty-seven per cent, the soldier was evacuated to a general hospital for treatment, for no specific medication was available at Mindoro. This single case made clear the necessity of conducting an immediate survey of all the hospital's personnel.

SURVEY OF ALL PERSONNEL

An attempt was first made to discover contact areas on Leyte in which potential infection with *S. japonicum* was likely. When the organization was staged on Leyte (November, 1944), it was bivouacked about three miles north of a native village immediately adjacent to a large rice paddy. A detachment of fifty-four men of the organization was stationed at another village, 20 miles away, and in order to reach this village they had had to traverse a fresh-water stream which was known to harbor a species of snails recognized as the intermediate host for *S. japonicum*. No snails of this species had ever been found in the vicinity of the bivouac area of the parent organization (6), but it was realized that the great majority of enlisted men, and many of the officers, visited back and forth between the two areas on various occasions, using a route which necessitated wading either in at least one infested river or several rice paddies near the endemic village. This was considered important in view of the recognized ability of the

cercariae of *S. japonicum* to penetrate the unbroken skin within a very few moments after contact, so that even a single exposure in the infested waters had to be considered as potentially sufficient for infection.

The survey carried out in April, 1944, brought to light thirty-four cases diagnosed as Asiatic schistosomiasis out of a total strength of one hundred and seventy-seven men. Of these 34, twenty-five had been in the detachment of 54 stationed in the endemic area, and five had had occasion to wade the infested stream repeatedly. The four remaining cases were in men who had made frequent trips between the detachment and the main body, but without crossing the infested river, and travelling only through adjacent rice paddies. These paddies were flooded several times when the river overflowed its banks: The events described occurred during the rainy season on Leyte. The men of the detachment of 54 had to cross the river at least twice daily where it varied in depth from about six inches to two feet. All of the men firmly denied that they had bathed in the infested, or in any other stream. For the first week of staging on Leyte, well water was used for bathing, and all drinking water was hyperchlorinated. After the first week, hyperchlorinated water was also used for bathing purposes.

STOOL EXAMINATIONS

The stools of 177 officers and enlisted men were examined. One specimen of each individual was studied, except for all of the men in the detachment of 54 and all others who had had frequent contact with the known infested waters. In all of these cases a minimum of two stools was required. Since the hospital at the time of the survey was operating at twice its rated bed capacity and the laboratory was already strained to keep up with the requirements of the wards, direct smears were taken of the great majority of stools. In only a few cases were stools searched for ova by the sedimentation method. Strands of mucus in the stools, if present, were found to be the most fruitful sources of ova. The initial stool studies resulted in twenty-two positive smears. Five positive smears were obtained from mucus secured by proctoscopy of men with positive proctoscopic findings and previous negative stools. Seven men had proctoscopic lesions typical of schistosomiasis and were so diagnosed; in six of these, subsequent stool examinations were positive for ova of *S. japonicum*. One individual had four consecutive negative stools after proctoscopic examination revealed lesions typical of schistosome infection, as well as an eosinophilia of 12 per cent, but he was returned to the United States on rotation before further stool studies could be done. He is not included in this series.

The stool survey also revealed eleven cases of ascaris infestation (two of them concomitant with schistosomiasis) and five of trichiuris infestation, all apparently symptom-free.

PROCTOSCOPIC EXAMINATIONS

Proctoscopic examination was carried out in only 148 persons because 29 men of the organisation were transferred out on rotation or for other reasons before the

survey was begun. Because the nurses and American Red Cross field workers arrived at Leyte on a later date and had no history of exposure, no female personnel were proctoscoped.

In every case the rectum, rectosigmoid, and sigmoid were carefully scrutinized. All lesions found were in the rectosigmoid and sigmoid mucosa. Twenty-three individuals had definite ulcers, plaques, "blisters," and distended venules, as described above; four showed only a patchy, stellate distension of venules, but no ulcers; and seven were entirely normal. Five positive smears were obtained from ulcers in the sigmoid and the rectosigmoid mucosa on proctoscopy, and it seems most probable that many more positives would have been secured if biopsy of typical plaques and "blisters" had been practised routinely.

There were three cases featured by a diffusely hyperemic, granular, easily bleeding mucous membrane which was due to a *Shigella* infection proven by laboratory cultures. One of these also had lesions typical of schistosomiasis with ulcers larger than those previously described. This patient had a large "blood blister" in the sigmoid, and scrapings of the ulcer beneath this lesion contained ova of *S. japonicum*.

SYMPTOMS

Table 1 shows the distribution of symptoms in the twenty-three individuals who complained of symptoms. Eleven men were entirely symptom-free, and twenty-eight of the 34 cases diagnosed were able to continue at their tasks without interruption and were handled throughout as out-patients. Of the eight cases hospitalized for short periods, four were admitted for "fever of undetermined origin," two for "bloody diarrhea," and two for an acute infectious hepatitis.

When any symptoms at all were present, the most helpful in diagnosis were constipation, constipation alternating with diarrhea, myalgia of the neck muscles, mild arthralgia, urticaria, and anorexia. Such vague complaints as generalized malaise, diarrhea, abdominal cramps, backache, headache, and moderate loss of weight are extremely common findings in many other disorders frequently seen in this theater. No symptoms were considered significant if they had existed before the patient arrived at Leyte. Certainly the great majority of the enlisted men and officers had had at least one bout of dysentery while the organization was staged at Leyte, so that this symptom was not considered as specific. Those cases listed under "Diarrhea" in the table had had at least two attacks of unusual diarrhea at Leyte. All of the men in whom a diagnosis was possible had also lost from five to twenty pounds within six months. Symptoms referable to the nervous system were not considered particularly significant because the great majority of exposed personnel had been overseas at least two years under frequently trying circumstances, and it was not felt that their minor psychopathologic aberrations could be attributed to any factor so mild as the recently acquired and currently symptomless infection they were proven to have. The four men who manifested urticaria had never before had any symptoms of allergic sensitivity.

TABLE 1
Findings of the 23 cases with symptoms

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Anorexia		X		N	X	X	X	X	X			X	X	X	X				N	X	X		
Diarrhea	X					M			M		X	M		M	X		X			X			X
Constipation		X				X		X		X												X	
Constipation alternating with diarrhea			X	X			X						X	X	X	X							
Abdominal Pain				X									X	X	X	X		X					X
Headache	X				X		X								X								
Myalgia of Neck	X			X		X														X	X	X	X
Back ache (Lumbar or Sacroiliac)		X	X	X	X	X														X	X	X	X
Arthralgia			X					X							X								
Cough	X						X																
Hospitalization (Other than Diarrhea)						F U O		F U O					F U O		F U O						H	H	II
Urticaria						X				X					X								

White Blood Count at Onset of treatment	White Blood Count (per mm ³)													
	10050	7650	5500	6500	7300	12800	11200	8100	5010	5700	5150	6280	7600	7950
Per cent Eosinophiles	11	12	1	1	0	0	12	20	0	1	10	1	6	11
Proctoscopic Findings	+	+	+	+	+	+	+	+	+	+	+	+	+	+

There were 11 cases with no complaints.

M—Melena

N—Nausea

FUO—Fever Undetermined Origin

H—Hepatitis

PHYSICAL FINDINGS

There was an almost disquieting absence of positive signs except for the laboratory findings, which the writers take as all the more reason for alertness and awareness of the possibility of schistosome infection in individuals returned to the United States from the Western Pacific Theater, and especially from regions already known to be endemic. The possibility of infection in areas formerly regarded as free from schistosomiasis must also be borne in mind, as has been demonstrated by Hunter in his studies of native populations in such communities, some of them here on Mindoro.

Except for five cases, the physical findings were essentially negative. In four cases the liver edge was barely palpable and tender to touch, and in one case the liver was enlarged approximately two centimeters below the costal margin and the spleen was palpable. In a number of cases, vague, irregular, fleeting tenderness was found on abdominal palpation. In Table 1 it will be seen that symptoms, the percentage of eosinophile, and the proctoscopic findings are not always correlated.

TABLE 2

PERCENTAGE OF EOSINOPHILES	NUMBER OF CASES
16-20	2
10-15	11
6-9	6
0-5	15

Leukocyte counts before treatment was undertaken ranged from 4,800 to 14,950. The eosinophile counts are tabulated in Table 2. All but six cases had complete blood counts a few days before treatment was begun. These six men had their initial blood counts during the first week of treatment. The highest eosinophile count was 20 per cent, which, to be sure, is not very high in the usual very active cases; but this degree of eosinophilia would perhaps be in accordance with the mildness of the symptoms and the short duration of the infection.

TREATMENT

No treatment was available at first at Mindoro until urgent requests to the Theater Surgeon were made. A supply of Fuadin was obtained, and the 34 patients diagnosed as positive were given a course of forty-five cc over a period of 17 days. It should be noted that the majority of patients reacted, late in the course, to the medication. The commoner symptoms were nausea, abdominal cramps, and scattered pains which suggested arthralgia or myalgia of brief duration. These reactions were for the most part inconsequential, and most of the men were able to continue at their assignments. Five men were hospitalized for three or four days. Two men had chills and fever, and seven complained of nervousness and insomnia, which, in several cases, was ascribed to arthralgia.

FOLLOW-UP CARE

At the conclusion of the standard Fuadin treatment, two consecutive stools were obtained from thirty-three patients. One patient had degenerate ova in the first stool, but two subsequent stools were negative for ova. All the other men had negative stools.

All positive cases were proctoscoped at from two to four weeks following the completion of treatment. In almost all cases the lesions had healed, and a few presented healing granular surfaces. Of two men who showed some improvement but who still had ulcers on the completion of a full course of Fuadin, one was returned to the United States on rotation without follow-up, and one, on proctoscopy six weeks after treatment, showed progressive healing except for four minute ulcers. He was evacuated to a general hospital at Leyte, where the diagnosis of schistosomiasis was confirmed. This man has since been evacuated to the United States for further observation and treatment.

Treatment in general, did not, to a significant degree alter, the eosinophile count one week after treatment was completed, although the count dropped in one case from 20 per cent to 4 per cent, and in the other from 10 per cent to 1 per cent. After one month of treatment, 19 of the 34 cases admitted marked improvement or entire amelioration of their symptoms. In only four cases was there no improvement one month after the completion of treatment.

Two months after treatment was completed, two consecutive stools were studied, using the sedimentation method, from twenty-three patients of this series. All were negative for ova of *S. japonicum*. The other eleven men had by this time left the organization, so that no follow-up was possible. Complete blood counts made two months after treatment revealed a marked reduction in eosinophilia in all cases. All the patients, except the one who was evacuated, have been on full duty.

SUMMARY

A stool and proctoscopic examination was carried out on one hundred and seventy-seven officers and enlisted men of a medical installation in the Western Pacific Theater. Thirty-four cases of Oriental schistosomiasis were found. There were eleven cases with *Ascaris lumbricoides* and five cases with trichuriasis infestation.

The diagnosis was made by finding the ova of *Schistosoma japonicum*. Immature and degenerative ova were also found in a number of cases. The ova were found in the stool specimens or on direct smear of the ulcers during proctoscopic examination. Twenty-three cases had proctoscopic findings indicative of schistosomiasis.

All of the 34 cases remained on full duty and had no serious disabling symptoms. One other member of the organization was hospitalized because of schistosomiasis prior to the survey and is not included in this group.

The excellent response of thirty-two cases to Fuadin is probably due to the mild pathological picture of the disease in this group, as Fuadin was not very

successful in the treatment of the usual active case of *Schistosomiasis japonica* (7, 8).

The authors agree with Bercovitz's opinion that there is a large group of individuals with schistosomiasis who show few or no symptoms. It is suggested that further surveys of organizations known to have lived in endemic areas would bring to light many additional cases of silent schistosomiasis in a relatively early stage of mild infection, before a heavy invasion of the liver has occurred (3). The importance of prompt and intensive treatment to prevent irreversible liver damage cannot be exaggerated.

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ARTHROPODS OF SANITARY IMPORTANCE IN THE REPUBLIC OF NICARAGUA, CENTRAL AMERICA¹

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The occurrence, distribution, and life histories of the arthropods of sanitary importance in Nicaragua are little known, and the status of many in relation to the health of man and domestic animals is in doubt. This kind of information should be available to serve as a basis for the formulation of programs for the control and eradication of arthropod-borne diseases. Since they are widely distributed in North, Central, and South America, these diseases are an immediate and mutual concern of all Americans. The nature of the problem therefore demands hemispherical planning and control.

An assignment to serve as medical entomologist and malaria control officer in connection with United States Naval interests in Central America, provided an opportunity for the writer to visit certain parts of Nicaragua to observe and study arthropods of medical importance. The purpose of this paper is to present information obtained on that occasion.

Reference is made to the map which shows the localities where observations were made. The survey extended from 26 July to 29 September (1943). Previous visits had been made to Corinto, Realejo, Cosiguina, and Fonseca, as noted below. Climatic and topographic conditions varied widely in the different places visited.

The annual rainfall along the Pacific coast varies approximately between 40 and 80 inches. Almost all of this falls between May and November, the normal rainy season.

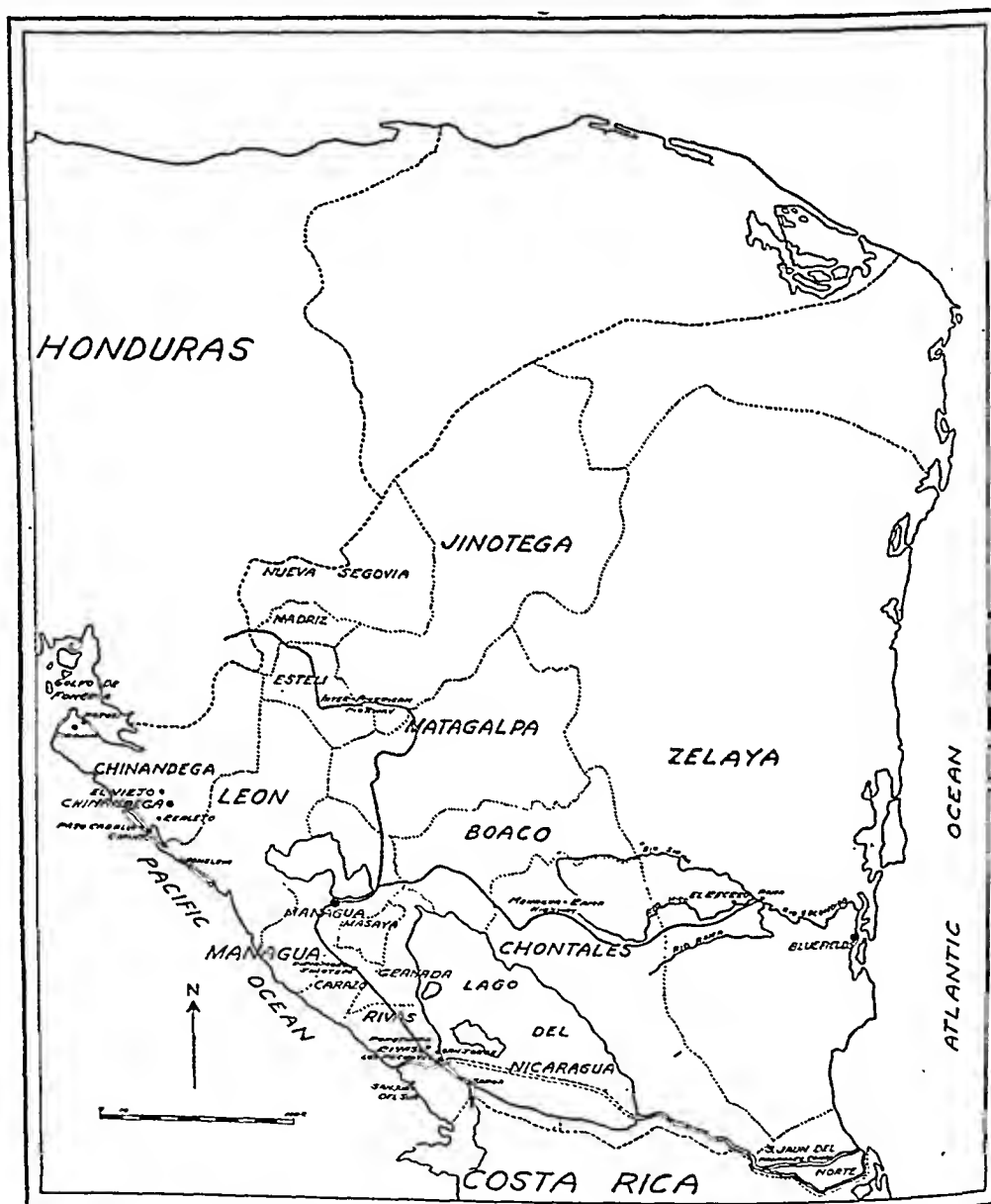
Records were obtained in Corinto, on nearby islands, and on nearby peninsulas of the mainland at various times between 7 November 1942 and 25 April 1943. This period was dry except for a very little rain that fell during November. Some pools remained from previous rains, and in these *Anopheles albimanus* bred in great numbers.

A visit was made to the territory in the general vicinity of Realejo, Department of Chinandega, in November. Although no rain had fallen during the past few weeks the rainy season marshlands were still marshy and mosquitoes were breeding prolifically in all standing water. The visit to the Department of Chinandega at Cosiguina, Fonseca, and Potosi was made in mid-July, and the visit in the vicinities of Chinandega and El Viejo was made during the first part of August. No rain had fallen since the previous rainy season. Certain marshes, particularly spring fed marshes, pools, and sluggish streams provide mosquito breeding places even during the dry seasons.

¹ The expressions in this paper are the private ones of the author and are not intended to represent official views of the Navy Department.

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The visit to the Department of Rivas was made during the latter part of September, shortly after the start of the seasonal rains in the vicinity of Rivas, Popoyuapa, San Jorge, Los Pochotes, and Sapoa. Perpetual marshes and sev-



OUTLINE MAP OF THE REPUBLIC OF NICARAGUA, SHOWING PLACES VISITED DURING A SURVEY OF THE ARTHROPODS OF SANITARY IMPORTANCE

eral sluggish streams that are obstructed at their outlets in Lake Nicaragua by sandbars, permit year-around production of mosquitoes. Extensive breeding immediately follows the beginning of rains and the formation of new breeding situations. No significant amount of rain had fallen in San Juan del Sur. Here *Anopheles* breeding (*Anopheles albimanus* and *Anopheles pseudopunctipennis*

pseudopunctipennis) was largely confined to an impounded estuary and to the edges of the stream which feeds the estuary.

Diriamba and Jinotepe, Department of Carazo, were visited during the last week of September. This comparatively high area was very dry.

The Department of Zelaya was visited in the vicinity of Bluefields, Rama, and El Recreo during August, immediately following the height of the rainy season. Moderate rains fell throughout the month. This area receives a yearly average of 150 inches of rain which falls throughout the year. The heavier rains usually occur during July. February is said often to be rainless. *Anopheles albimanus* breeds prolifically in the extensive marshes in the vicinity of Bluefields, and in grassy streams and marshy places in and immediately adjacent to the city.

Primary consideration was given to the investigation of the species that are proven vectors or that are causative agents of prevailing human diseases. Attention was given next to those species that are probable or possible transmitters or causative agents of diseases of man and domestic animals, or that are pests. Associated species, many of which are parasitic or predacious, and taxonomically closely related species, knowledge of which is essential in investigational and control operations, frequently were taken incidentally during searches for species of major sanitary importance. The species of sanitary importance occur as elsewhere in various degrees of association with man and with domestic animals. They frequent human habitations. They visit body wastes, garbage, and other possibly infected materials and, significantly, human food. Very many live and breed upon the animal or person, bite or sting, or pierce the skin for the purpose of obtaining blood.

Information in regard to the prevalence of the specific arthropod-borne diseases which are briefly discussed below was obtained from conversations with health authorities, local physicians, stockmen, and veterinarians, and to a limited extent from literature.

The high incidence and relatively great importance of malaria places *Anopheles albimanus*, the principal vector, as the most injurious arthropod of Central America. *Anopheles albimanus* is widely distributed and breeds in abundance under the favorable environmental conditions that prevail throughout its range. *Anopheles pseudopunctipennis pseudopunctipennis* also might be an important vector. It was found only in Rivas Department, but probably has much wider distribution.

Dermatobia hominis (Linn.), the human botfly and the "tór-salo" of cattlemen, is widespread, especially in the higher lands of Matagalpa, Esteli, Boaco, Chontales, Rivas (in the vicinity of Sapoá), and Zelaya. It is especially prevalent along the rivers of Zelaya, where the Indians frequently are victims. The tór-salos are said to disappear in cattle brought to the lower, hotter climate of Chinandega from the higher land of Chontales. Tór-salos are a major pest of cattle and cause much loss in the value of hides. Man frequently is infected. Various species of *Psorophora*, *Aedes*, *Culex*, *Wyeomyia*, *Anopheles*, *Antheromyia*, *Stomoxys*, *Synthesiomyia*, *Musca*, *Sarcopromusca*, *Limnophora*, *Peridita*, and *Sarcophaga*, and the tick *Amblyomma cajennense*, are proven vectors which are numerous and widely distributed in Nicaragua. Considering the diverse habits

of the known carriers, it is probable that almost any bloodsucking arthropod or almost any insect that occasionally alights upon animals might serve as a carrier.

Myiasis of various other kinds occur frequently in man and in livestock. *Musca domestica*, *Cochliomyia macellaria*, and species of *Calliphora*, *Lucilia*, *Sarcophaga*, *Fannia*, Syrphidae and Stratiomyidae are of common occurrence.

The incidence of amoebic dysentery is reported high. The presence of bacillary dysentery is acknowledged but specific diagnoses as such are infrequent. Typhoid apparently occurs in most parts of the country. Stored water in pilas, above ground cisterns, and barrels, during the dry season, are probably important sources. Mechanical carriers of these three intestinal diseases are several kinds of flies that frequent human habitations, human excrement, and human food. Among these are the cosmopolitan *Musca domestica* and species of *Fannia*, *Sarcophaga*, *Lucilia*, *Cochliomyia*, *Ophyra*, *Synthesiomysia* and *Sarcophagula*.

American mucocutaneous leishmaniasis is reported to occur infrequently. However at least one case diagnosed as such occurred recently in Bluefields, Zelaya Department. Environmental conditions are favorable for the breeding of species of the genus *Phlebotomus*, among which are suspected vectors. Any of several other bloodsucking flies that occur in the vicinity might be vectors.

The last epidemic of yellow fever occurred between 1918 and 1921. Provision should be made nevertheless for the possibility that it again might be introduced, since the sole urban vector, *Aedes aegypti*, breeds abundantly in all the localities that were visited. Also any of the large number of jungle-inhabiting bloodsucking mosquitoes might be capable of transmitting the disease. Such species are suspected vectors in endemic areas of South America and Africa where more than twenty have been proven capable of transmitting the virus by bite.

Wuchereria bancrofti is endemic in other parts of Central America and some form or forms of filariases are reported to occur in Nicaragua. Any of the large number of species of bloodsucking mosquitoes found in Nicaragua might serve as intermediate hosts. *Culex fatigans*, *Aedes aegypti*, and *Anopheles albimanus*, proven vectors, breed prolifically in all the localities that were visited.

Conditions appear favorable for epidemics of dengue. The only known vector in the Western Hemisphere, *Aedes aegypti*, is widely distributed and breeds abundantly. *Aedes taeniorhynchus*, a suspected vector, is also widely distributed.

Environmental conditions including the presence of arthropod vectors, appear favorable for the existence of the following diseases, but their occurrence in Nicaragua is not known to the writer.

Onchocerciasis occurs nearby in Guatemala. Species of Simuliidae (probable vectors) occur in Nicaragua.

Cases of *Dipylidium caninum* (Linn.), the double-pored dog tapeworm, a common parasite of the dog, are occasionally reported in humans, especially children. The intermediate hosts, the biting dog louse, *Trichodectes canis*, the fleas found on dogs, *Ctenocephalides canis*, *Ctenocephalides felis*, and *Pulex irritans*, occur in abundance. Dogs are exceedingly numerous.

Yaws and pinta (carate) are recorded as occurring in Central America.

Species of *Hippelates*, known vectors of yaws, occur in Nicaragua. *Simulium* species which with *Hippelates* are suspected vectors of pinta, also occur.

Conjunctivitis might be spread by *Hippelates pusio* which is found in Nicaragua.

Bloodsucking diptera that are possible disease carriers as well as pests are represented by species of Simuliidae, Tabanidae, Culicidae, Heleidae, particularly *Culicoides furens*, and Muscidae, particularly *Stomoxys calcitrans* and *Siphona irritans*. Many of the species in these groups occur in unusually large numbers and often are vicious biters. The annoyance and irritation disturb sleep and rest and thus indirectly reduce endurance to a marked degree. The Tabanidae, suspected transmitters of anthrax and tularemia, are frequently very numerous and severely attack both livestock and man. *Aedes taeniorhynchus*, a vicious biter, is one of the most numerous of the pest mosquitoes. *Aedes aegypti* and *Culex fatigans* were breeding prolifically in all cities and villages that were visited. *Aedes taeniorhynchus* and *Aedes aegypti* are two of the numerous species suspected of transmitting equine encephalomyelitis in North America. *Culicoides furens* is a very severe pest throughout the coastal regions of Central America. *Stomoxys calcitrans* besides being a severe biter and bloodsucker of man and livestock, is also suspected of carrying anthrax. *Siphona irritans* is numerous and a severe biter and bloodsucker of cattle and horses. Very many biting species that occur in smaller numbers are collectively very annoying both in cities and in the jungle. Any of these bloodsucking insects are suspects when vectors of diseases of man or livestock are sought.

The common external parasites: mites and ticks, biting and sucking lice, and fleas, are abundant. Ticks are extremely numerous in cattle country. Dipping, practiced a few years ago, was not carried on about Chinandega during the war years.

LIST OF ARTHROPODS WITH GEOGRAPHICAL DISTRIBUTION RECORDS AND NOTES^{3,4}

Class ARACHNIDA :

SCORPIONIDA (Scorpions)

Centruroides sp. Occur in all types of human habitations and outside. Frequently found in bed clothing, clothing, shoes. Sting is very painful.

CHINANDEGA. Chinandega, Fonseca, Potosi. RIVAS. Los Pochotes, San Juan del Sur.

ARANEIDA (Spiders)

Latrodectus geometricus (C. L. Koch) Very numerous on the underside of furniture and on inner walls of houses. No instance of biting of human beings came to the writer's attention. Reported in the literature to be venomous, but its bite is said to be less serious than that of *Latrodectus mactans* (F.), the black widow spider. CHINANDEGA. Corinto.

³ In the distribution records, Departments are given in capitals; Localities in lower case.

⁴ All species for which the geographical distribution records give Fonseca or Cosiguina as a locality were taken by Commander John D. DeCoursey.

Eurypelma seemanni (Pick.-Camb.) This tarantula was taken from a hole in the ground. Commonly known as Araña pica caballo. The native Indians regard them as dangerous. The belief is general that this and similar species are responsible for a condition that leads to the loss of hoofs by horses. CHINANDEGA. El Viejo.

ACARINA (Mites and Ticks)

Sarcoptidae

Psoroptes equi (Her.) Found in ears of horses. CHINANDEGA. El Viejo, Fonseca.

Ixodidae (Unidentified larvae) Found at base of scales of an iguana. CHINANDEGA. Fonseca.

Rhipicephalus sanguineus (Latr.) Found on dogs. CHINANDEGA. El Viejo. RIVAS. Rivas, San Juan del Sur.

Boophilus annulatus australis (Ful.) Cattle were heavily infested. Several were found on two horses. CHINANDEGA. Chinandega, El Viejo, Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, Rama.

Amblyomma (Unidentified species) Found on man, cattle, horses, pigs, iguana, armadillo. CHINANDEGA. Chinandega, Fonseca, Realejo. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, Rama, Rio Escondido.

Amblyomma cajennense (F.) Found on man, cattle (heavily infested), horses, pigs. CHINANDEGA. Chinandega, El Viejo, Potosi, Corinto. ZELAYA. Rama.

Dermacentor nitens (Neum.) Horses and mules were heavily infested. Found also on cattle. CHINANDEGA. Chinandega, El Viejo, Fonseca, Cosiguina. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, Rama, El Recreo.

Class HEXAPODA

ORTHOPTERA

Blattidae (Roaches)

Periplaneta americana (L.) Present in numbers in human habitations. RIVAS. San Juan del Sur.

Periplaneta australasiae (F.) Numerous in human habitations. CHINANDEGA. Chinandega. RIVAS. San Juan del Sur. ZELAYA. Bluefields, Rama.

Blaberus giganteus (L.) Occurred in numbers at base of hollow tree. Few found in human habitations with dirt floor, in jungle. CHINANDEGA. El Viejo. RIVAS. San Juan del Sur.

Blaberus discoidalis (Serv.) Found about lumber mills and about primitive human habitations. CHINANDEGA. Chinandega.

Panchlora sp. Flying roach found in human habitation. CHINANDEGA. Chinandega.

Pycnoscelus surinamensis (L.) A flying roach, attracted to light. CHINANDEGA. Chinandega.

Supella supellectilium (Serv.) Present in numbers in human habitations. (Recently introduced into North America). CHINANDEGA. Chinandega.

Necostylopyga rhombifolia (Stoll) Found in human habitation.

ODONATA

Aeshnidae (The Aeshnid Dragon-flies) The naiads occurred in large numbers and were predacious on mosquito larvae in grassy pools. RIVAS. Popoyuapa.

MALLOPHAGA (The Biting Lice)

Menopon gallinae (L.) Found on domestic chicken. RIVAS. Rivas.

Heterodorus longitarsus (Piag.) Found on dogs and on one horse. CHINANDEGA. El Viejo, Cosiguina. RIVAS. San Juan del Sur. ZELAYA. Bluefields.

Oryliperus angularis (Peters) Found on domestic chicken. Rare according to Dr. H. E. Ewing. RIVAS. Rivas.

Trichodectes canis (Deg.) Dogs were infested. ZELAYA. Bluefields.

Felicola subrostrata (Nitz.) Found on cats. ZELAYA. Rama.

ANOPLURA (The Sucking Lice)

Haematopinus adventicius (Neum.) Pigs were heavily infested. CHINANDEGA. El Viejo. RIVAS. San Juan del Sur.

Pediculus humanus americanus (Ewg.) Found in limited numbers on heads of children and adults. CHINANDEGA. Chinandega, El Viejo, Fonseca, Cosiguina, Corinto. RIVAS. San Juan del Sur.

Phthirus pubis (L.) Pubic region of man. Reported to be common. CHINANDEGA. Corinto.

HEMIPTERA (The Bugs)

Belostomatidae

Lethocerus annulipes (H. - S.) Predacious on mosquito larvae. Known to bite man severely. CHINANDEGA. Fonseca.

Cimicidae (The Bedbugs)

Cimex hemipterus (F.) Numerous in human habitations. CHINANDEGA. Chinandega, Fonseca, Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields.

COLEOPTERA (The Beetles)

Dytiscidae

Laccophilus sp. Larvae were predacious on mosquito larvae in temporary rain pools. RIVAS. Rivas.

Hydrophilidae

Hydrous sp. Larvae may be predacious on mosquito larvae. RIVAS. Rivas, Popoyuapa.

Tropisternus sp. Larvae may be predacious on mosquito larvae. RIVAS. Rivas, Popoyuapa.

Hydrophilus ater Oliv. CHINANDEGA. Fonseca.

Scarabaeidae

Pelidnota strigosa Lap.

Athyreus sp.

Pinotus carolinus (L.) CHINANDEGA. Chinandega.

DIPTERA (The Flies)

Tipulidae (The Crane Flies)

Limonia (Geranomyia) sp. One adult found in horse stable. MANAGUA.

Managua.

Orimarga sp. Three adults taken from lily plants. ZELAYA. Rama.

Polymeda spp. Eight adults were taken; one from lily plant, seven at light. ZELAYA. Rama.

Simuliidae (The Black Flies)

Simulium pulverulentum (Knab.) Occurred in enormous numbers. The larvae and pupae formed a soft mat on the wooden floor of a water chute, to which they were attached. CHINANDEGA. Chinandega.

Simulium quadrivittatum Loew. Adults taken while biting man in jungle. ZELAYA. Rama.

Tendipedidae (The Midges)

Pentaneura sp., *Tendipes* sp. Attracted to electric lights in large numbers. Apparently cross Lake Nicaragua from Chontales. *Tendipes* sp. adults found on damp inside walls of building and at lights. CHINANDEGA. Chinandega. RIVAS. Los Pochotes. ZELAYA. Rama.

Spaniotoma sp. One adult taken about man. ZELAYA. Rama.

Heleidae (The Biting Midges)

Forcipomyia sp. One adult was captured at light. ZELAYA. Rama.

Atrichopogon sp. One found in vegetation about water hole. CHINANDEGA. Fonseca.

Dasyhelea sp. Pupae found in leaf-base of lily plant. ZELAYA. Rama.

Culicoides furens (Poey) Adult females taken about man and at light. Most common Central American sandfly pest. CHINANDEGA. Fonseca, Corinto.

Culicoides sp. Adult females taken about man in jungle, and at light. Very severe biters. CHINANDEGA. Corinto. ZELAYA. Rama, Bluefields.

Heteromyia sp. Pupae very numerous at surface of water in marsh. Adults bred out. ZELAYA. Bluefields.

Bezzia sp. Pupa taken with mosquito larvae from tree hole. Adult bred out. RIVAS. Rivas.

Psychodidae (The Moth Flies)

Telmatoscopus albipunctatus (Will.) Large numbers of larvae and pupae were at surface of muddy rainwater in covered tub partly filled with soil. Adults bred out. ZELAYA. Bluefields.

Psychoda spp. Large numbers were attracted to lights. ZELAYA. Rama.

Culicidae (Mosquitoes and near allies)

Chaoborus brasiliensis (Townsend) Adults were attracted to electric lights in large numbers. Enormous swarms appear suddenly over Lake Nicaragua, coming from the direction of the Department of Chontales. One such swarm was observed to reach the western shore of the lake one hour after sunset. RIVAS. Los Pochotes.

Corethrella sp. Larvae were numerous in water held by the leaf-bases of small epiphytic bromeliads. Adults bred out. ZELAYA. Rama.

Limatus durhamii (Theobald) Larvae were obtained from water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Wyeomyia celaenocephala (Dyar and Knab) One adult was captured while about person. ZELAYA. Rama.

Wyomyia circumcincta (Dyar and Knab) Larvae were taken from water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Phoniomyia lassalli (Bonne-Wepster and Bonne) Larvae were taken from water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Psorophora confinnis (Lynch Arribáizaga) Larvae were found in the stagnant water of pools and ox-cart wheel ruts exposed to the sun. Some breeding places were grassy, others had little or no vegetation. RIVAS. Rivas, Popoyuapa, San Jorge.

Psorophora ferox (Humbolt) Adults were captured while about man. CHINANDEGA. Realejo.

Psorophora howardii (Coquillett) Larvae were found in the stagnant water of pools and ox-cart wheel ruts exposed to the sun. Some breeding places were grassy, others had little or no vegetation. RIVAS. Rivas, Popoyuapa, San Jorge.

Haemagogus anastasionis (Dyar) Larvae were taken from water in a tree hole. RIVAS. San Juan del Sur.

Haemagogus equinus (Theobald) One larva was found in water from a hole in a log. Two adults (females) were taken about man. RIVAS. Rivas, San Juan del Sur.

Haemagogus chalcospilans (Dyar) Larvae were found in a half-coconut shell which was shaded by coconut palms and banana trees. RIVAS. San Juan del Sur.

Aedes aegypti (Linnaeus) Larvae occurred in rainwater tanks, rain-barrels, open drums, concrete basins, tubs, unused boats, and in many other kinds of water containers about human habitations. They were found also in small pools of water in the bottom of shallow wells during dry periods. The usual shallow well is about two feet square and five feet in depth. It is lined with tin or wood, and open at the top. Adults occurred in all types of human habitations, about man by day and by night, and in the dark or by bright electric lights. CHINANDEGA. Chinandega, Paso Caballo, Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, El Bluff, Rama, El Recreo.

Aedes angustivittatus (Dyar and Knab) One adult was taken in a fresh water marsh. ZELAYA. Rama.

Aedes euplocamus (Dyar and Knab) Larvae were taken from shaded water in ox-cart wheel ruts. CARAZO. Jinotepe.

Aedes terrens (Walker) Several larvae were obtained from water in tree rot holes, a few from a small, wooden trough, and others from relatively clean shaded water in a concrete basin. One female was taken while biting man. CHINANDEGA. Consiguina, Corinto. RIVAS. Rivas, San Jorge, Los Pochotes, San Juan del Sur.

Aedes taeniorhynchus (Wiedemann) Larvae were obtained from brackish water pools on fringe of tidal marsh, from stagnant fresh water pools in river bed (dry season), from upland fresh water pools, from very muddy water of drying pool in pasture, and from grassy ditches. Breeding places were in par-

tial shade or were fully exposed to the sun. Adults were taken about man and about horses, in grass and weeds near breeding places, in human habitations, and in the open, at all times of the day and night. CHINANDEGA. Chinandega, El Viejo, Realejo, Corinto. RIVAS. San Jorge, San Juan del Sur. *Deinocerites epitedeus* (Knab) Numerous adults were trapped from crab holes near a tidal marsh. They were associated with *Deinocerites pseudus* adults. CHINANDEGA. Corinto.

Deinocerites pseudus (Dyar and Knab) One pupa (adult bred out) was obtained from water in a well. Adults (all females) were taken from crab holes, and in barracks. Crab hole specimens were numerous and associated with *Deinocerites epitedeus*. CHINANDEGA. Corinto.

Culex corniger (Theobald) Larvae were found in rainwater containing decaying leaves in an unused, rotting boat under a tree; in stagnant pools of a river bed; and in a tub of rainwater (numerous). Two larvae were obtained from the water in a vat in which skins are washed during the tanning process. CHINANDEGA. Chinandega. ZELAYA. Bluefields.

Culex coronator (Dyar and Knab) Larvae were found in the relatively fresh, clean water of drums, concrete and wooden receptacles, small pools, ox-cart wheel ruts, hoof prints, and fresh water marshes; in water containing much organic matter in borrow pits and in rainwater containing decaying leaves in a rotting boat; in stagnant pools of a river bed, and in stagnant water of an unused dipping vat; and in the foul water of pools, of a scale pit containing decaying vegetable matter, and of a wooden watering trough for chickens. The water of the breeding places was with or without vegetation and the breeding places were in shade or sun. CHINANDEGA. Chinandega, El Viejo, Realejo, Corinto. CARAZO. Diriamba, Jinotepe. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, El Recreo.

Culex daumastocampa (Dyar and Knab) Larvae were taken from the water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Culex declarator (Dyar and Knab) Larvae were found in stagnant ground-pools which were exposed to the sun and possessed little or no vegetation. RIVAS. Rivas.

Culex erraticus (Dyar and Knab) Larvae were taken from water exposed to the sun in a borrow pit and in hoof prints. Also they were taken from vegetation at the edge of streams and were found in mats of surface vegetation in an estuary. CHINANDEGA. Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. El Recreo.

Culex fatigans (Wiedemann) (*C. quinquefasciatus* Say) Larvae were found in the relatively clean water of artificial containers about human habitations, such as wooden and concrete watering basins, cuspidors, barrels, open drums, and wells; in water containing much organic matter as found in an unused rotting boat and in watering basins; and in very foul water in seepage pits, latrines and artificial containers. Very large numbers of larvae were found in the foul water held by the trenches of latrines in cities as well as in small villages. Adults were taken about man in human habitations and out-of-

doors, and on damp inside walls of buildings. CHINANDEGA. Chinandega, Corinto. CARAZO. Diriamba, Jinotepe. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, Rama, El Recreo, Rio Escondido.

Culex mollis (Dyar and Knab) Larvae were obtained from water in an unused dipping vat. CHINANDEGA. El Viejo.

Culex nigripalpus (Theobald) Larvae were found in a tub of rainwater, in natural pools of clean water with vegetation, in the muddy water of recently dug holes without vegetation, in ox-cart wheel ruts, and in the foul water of a pit. Larvae were taken in water fully exposed to the sun as well as in completely shaded water. RIVAS. Rivas, Popoyuapa, San Jorge, Sapoá. ZELAYA. Bluefields.

Culex pilosus (Dyar and Knab) Larvae were taken from water in a roadside ditch which was grown up in grass and other vegetation and contained dead leaves. ZELAYA. Bluefields.

Culex interrogator (Dyar and Knab) Larvae were plentiful in the stagnant water of an unused dipping vat. CHINANDEGA. El Viejo.

Cules imitator (Theobald) Larvae were numerous in water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Culex (Microculex) sp. Larvae were taken from the water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Aedeomyia squamipennis (Lynch Arribáizaga) Larvae were obtained from among plants at the edge of a stagnant stream. RIVAS. Rivas.

Orthopodomyia sp., perhaps *kummi* Edwards. Larvae were taken from water in a tree hole. CHINANDEGA. Cosiguina.

Megarhinus moclezuma (Dyar and Knab) Four pupae were obtained from rainwater in a barrel. Adults were reared from three pupae. (Collection by DeCoursey). CHINANDEGA. Corinto.

Uranotaenia geometrica (Theobald) Larvae were taken from the water in a roadside ditch which was grown up in grass and other vegetation and contained dead leaves. ZELAYA. Bluefields.

Uranotaenia lowii (Theobald) Larvae were obtained from a grassy ground pool. RIVAS. Rivas.

Anopheles albimanus (Wiedemann) Larvae were found in the fresh water of pools, hoof prints, ox-cart wheel ruts, ditches, open trenches, borrow pits, fresh water and spring fed marshes, side pools of slow streams, matted surface growth of aquatic vegetation in a shallow, stagnant estuary, and in concrete vats. Usually they were found among vegetation, particularly grass and algae, but sometimes in water without vegetation. Invariably the breeding places were fully exposed to the sun. Adults were found in human habitations, including barracks, by day and by night. While usually resting by day and active at night, a few were observed to bite in shady places on very bright days. They also attack and bite under bright electric light. CHINANDEGA. Chinandega, El Viejo, Fonseca, Cosiguina, Potosí, Realejo, Corinto. RIVAS. Rivas, Popoyuapa, San Jorge, San Juan del Sur. ZELAYA. Bluefields, Rama, El Recreo.

Anopheles eiseni (Coquillett) A pupa from which a male emerged, was obtained from water of a rock hole in a rocky stream bed in dense shade. ZELAYA. Bluefields.

Anopheles neivai (Howard, Dyar and Knab) Larvae occurred abundantly in the water held by the leaf-bases of small epiphytic bromeliads. ZELAYA. Rama.

Anopheles neomaculipalpus (Curry) Larvae were found in grassy pasture pools fully exposed to the sun during the height of the rainy season. ZELAYA. Rama.

Anopheles pseudopunctipennis pseudopunctipennis (Theobald) Larvae occurred in large numbers in matted surface growth of aquatic vegetation in a shallow, stagnant estuary, in grassy pools, in pools with algae, and in shallow, slow streams and side pools of streams with vegetation. The breeding places were fully exposed to the sun, or shaded at most only part of the day. They were found in very warm shallow water exposed to the sun. Adult females were found in houses and in an open shed by Rio Merina. RIVAS. Rivas, San Juan del Sur.

Reference is made to table 1 which shows the frequency of association of the different species of mosquito larvae found on this survey. The table serves to suggest the possible existence of certain common environmental adaptabilities, but does not attempt to present the specific environmental conditions required by each species nor to show the common or overlapping conditions. The limited number of cases as well as the lack of detailed data on the specific environmental factors is too small to justify extended statistical treatment. Accurate knowledge of environmental requirements and adaptabilities, and of larval associations, has definite value in control operations that are directed against selected species.

Stratiomyidae (The Soldier Flies)

Artemita bicolor Kert One adult found about cattle. RIVAS. San Juan del Sur.

Tabanidae (The Horse Flies and Deer Flies, "Tábanos")

Chrysops melaena (Hine) Adult females were taken while biting horses in swampy jungle. CHINANDEGA. Cosiguina. ZELAYA. Rama.

Chrysops variegata (Deg.) Adult females were taken while biting horses in swampy jungle. CHINANDEGA. Cosiguina.

Chrysops mexicana (Kroeb.) Adult females were taken from horses on a forest trail in hills. ZELAYA. Bluefields.

Chrysops incisa (Macq.) Adult females were taken from horses and in the surroundings of horses on a forest trail in hills. ZELAYA. Bluefields.

Diachlorus jobbinsi (Fairch.) One adult female was taken from a horse on a forest trail in hills. ZELAYA. Bluefields.

Dichelacera analis (Hine) Numerous in jungle and woodland. Severe biter of man, horses and mules. ZELAYA. Bluefields, Rama.

Dichelacera marginata (Macq.) Adult females were taken from man and horse on a jungle trail. ZELAYA. Rama.

Tabanus sp., *curtus* group. One adult female taken while biting horse in forest. CHINANDEGA. Cosiguina.

Tabanus lincola carneus (Bell) Three adult females were taken in buildings (Corinto) and on a lily plant (Rama). CHINANDEGA. Corinto. ZELAYA. Rama.

Tabanus lincola stenoccephalus (Hine) Adult females were taken from a horse on a forest trail in hills. ZELAYA. Bluefields.

Tabanus albocirculus (Hine) One adult female was captured in the surroundings of man and horse in the jungle. ZELAYA. Rama.

Tabanus unistriatus (Hine) One adult female was captured in the surroundings of man and horse in the jungle. ZELAYA. Rama.

Tabanus oculus (Walk.) Adult females were caught while biting horses on forest trails. CHINANDEGA. Cosiguina. ZELAYA. Bluefields.

Leucotabanus leucaspis (Wied.) One adult female taken while biting horse in forest. CHINANDEGA. Cosiguina.

Psolidia ocellata (End.) Six adult females were captured about man and horse on forest trail in hills and on trail in jungle. ZELAYA. Bluefields, Rama.

Bombyliidae (The Bee Flies) (The larvae are parasitic upon many kinds of other insects.)

Anthrax sp. One found resting on ground, in sun. RIVAS. San Juan del Sur.

♀ *Phthiria sp.* ZELAYA. Rama.

Dolichopodidae (The adults are predacious upon smaller insects and mites.)

Pelastoneurus sp. Three found in vegetation about water hole. CHINANDEGA. Fonseca.

Syrphidae

Volucella sp. Larvae found in vat in which hides are treated in the process of making leather. CARAZO. Diriamba.

Mesogramma sp., probably *polita* (Say) Three adults about man. CHINANDEGA. El Viejo.

Tubifera sp. Larvae found in vat in which hides are treated in the process of making leather, and in horse trough. CHINANDEGA. Chinandega. CARAZO. Diriamba.

Otitidae

Acrosticta pallipes (Grimsh) One adult on horse in jungle. ZELAYA. Rama.

Drosophilidae (The Small Fruit Flies)

Drosophila melanogaster (Meig.) Taken at light. ZELAYA. Rama.

Drosophila repleta (Woll.) Common species in human habitations. CARAZO. Jinotepe. RIVAS. Rivas.

Chloropidae (The Frit Flies)

Hippelates pusio (Lw.) Adult flies very numerous about exposed raw beef and on saddle sore of horse. CHINANDEGA. Fonseca.

Muscidae

Musca domestica (L.) Common in human habitations and about horses and cattle. Breeding places are latrines, horse and cow manure, and garbage. CHINANDEGA. Chinandega. MANAGUA. Managua. CARAZO. Jinotepe. RIVAS. Rivas, San Juan del Sur.

[illegible]

Synthesiomys nudiseta (V. d. W.) Taken on exposed raw beef. CHINANDEGA. Fonseca.

Neomuscina sp. Adults about horses. ZELAYA. Bluefields.

Stomoxys calcitrans (L.) Common about cattle. ZELAYA. Bluefields.

Sarcopromusca arcuata (Tns.) Found about horses. ZELAYA. Bluefields.

Siphona irritans (L.) These flies are very numerous about cattle. RIVAS. San Juan del Sur. ZELAYA. Rio Escondido.

Anthomyiidae

Fannia sp. Found about human habitations and about horses. CHINANDEGA. Fonseca. RIVAS. Rivas, San Juan del Sur. ZELAYA. Rama.

Limnophora sp. Flies taken from vegetation about water hole and about human habitations and horses. CHINANDEGA. Fonseca. RIVAS. Rivas.

Ophyra aenescens (Wd.) Very numerous about human habitations. CHINANDEGA. Chinandega, Corinto. RIVAS. San Juan del Sur.

Calliphoridae (The Blowflies)

Cochliomyia macellaria (F.) Found about horses. ZELAYA. Bluefields.

Lucilia eximia (Wd.) Flies about exposed meat. CHINANDEGA. Fonseca.

Sarcophagidae (The Flesh Flies)

Metopia campestris (Fall.) Taken in horse pasture. CHINANDEGA. Fonseca.

Sarcophaga gulo (F.) Found in human habitations. CHINANDEGA. Corinto.

Sarcophaga xanthosoma (Ald.) Found in human habitations. CHINANDEGA. Fonseca, Corinto.

Sarcophaga aurigena (Tns.) Captured on jungle trail. ZELAYA. Rama.

Sarcophaga plinthopyga (Wd.) Occur in human habitations. CHINANDEGA. Fonseca. RIVAS. Rivas, San Juan del Sur.

Sarcophaga chrysostoma (Wd.) Adults found in vegetation about water and in human habitations. CHINANDEGA. Fonseca.

Sarcophaga occidua (F.) Occurred in large numbers in human habitations. Found in vegetation about pools and in horse pastures. CHINANDEGA. Fonseca. RIVAS. San Juan del Sur.

Larvaevoridae

Paraphasiopsis sp. One adult taken from a horse. ZELAYA. Bluefields.

SIPHONAPTERA (Fleas)

Pulex irritans (L.) Dogs were heavily infested. CHINANDEGA. Chinandega, El Viejo, Fonseca, Cosiguina, Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. Rama.

Ctenocephalides felis (Bouché) Dogs and cats were heavily infested. CHINANDEGA. Chinandega, El Viejo, Fonseca, Cosiguina, Corinto. RIVAS. Rivas, San Juan del Sur. ZELAYA. Bluefields, Rama.

Ctenocephalides canis (Curtis) Occurred on dogs and cats. CHINANDEGA. Cosiguina. RIVAS. San Juan del Sur.

HYMENOPTERA

Argidae

Caloptilia sp. RIVAS. Rivas.

Braconidae (The larvae are parasitic upon other insects.)

Iphiaulax sp. Found in weeds of horse pasture. CHINANDEGA. Fon-
ceca.

Ichneumonidae (The larvae are parasitic upon other insects.)

Pholocryptus pachymenac (Cr.) RIVAS. San Juan del Sur.

Psammocharidae (The Spider-Wasps)

Priocnemis fairchildi (Bks.) Found in jungle. ZELAYA. Rama.

Formicidae (The Ants)

Pseudomyrma gracilis (F.) var. Found on tree trunk. CHINANDEGA.
Fonceca.

Necoponera villosa (F.) Found in bromeliads. A very severe stinger. ZE-
LAYA. Rama.

Paraponera clavata (F.) Occur in large numbers in the jungle. Many found in
the decayed wood at the base of hollow trees. The sting of these ants is very
painful. ZELAYA. Rama.

Camponotus sciriceiventris (Guer.) Found on ground. CARAZO. Jinotepe.

Paratrechina longicornis (Latr.) Troublesome house ants. CHINANDEGA.
Chinandega.

Tapinoma melanocephalum (F.) Troublesome house ants. Destroyed dead in-
sects that had been collected for identification. CHINANDEGA. Chinan-
dega.

Vespidæ (The Typical Wasps)

Mischocyttarus pallidipectus (Sm.) Stinging wasp known locally as "avispa es-
panto muchacho". Found in house. RIVAS. San Juan del Sur.

Polistella picteti (Sauss.) Wasp known locally as "avispa saca sangre". RI-
VAS. San Juan del Sur.

Polistes sp. Stinging wasp known locally as "avispa corre cayote", "avispa
catarina", or "avispa caranera". Found in buildings. RIVAS. San Juan
del Sur.

Polistes carnifex (F.) Stinging wasp known locally as "avispa ogadora".
Found in buildings. RIVAS. San Juan del Sur.

Polistes canadensis erythrocephalus (Latr.) Stinging wasps found about human
habitations and about flowers. CARAZO. Jinotepe. ZELAYA. Bluefields.

Polybia sp. Several found on a sugar mixture. RIVAS. Rivas.

Synoecca surinama (L.) Stinging wasp, known locally as "Guitaron" from large
paper nest attached to high branch of tree. CHINANDEGA. Chinandega.

Parachartergus apicalis (F.) Stinging wasp known locally as "avispa corre cay-
ote". CHINANDEGA. Chinandega.

Sphecidae

Sceliphron assimile (Dahlb.) Stinging wasps known locally as "avispa carpen-
tera". Found in buildings. RIVAS. San Juan del Sur.

Trypoxylon spinosum (Cam.) Found in human habitation. Said to be harmless.
CHINANDEGA. Chinandega. RIVAS. San Juan del Sur.
Stictia signata (L.) CHINANDEGA. Corinto.

Apidae

Euglossa (Eulacma) surinamensis (L.) Numerous in seldom used ranch house.
CHINANDEGA. El Viejo.
Euglossa (Euglossa) cordata (L.) Captured in ranch house. A severe stinger.
ZELAYA. Bluefields.
Euglossa sp. Captured in house. A severe stinger.
Trigona sp. Stingless social honeybees that get in the hair and bite the scalp.
Captured in jungle. One nest found in tree hole. RIVAS. Rivas. ZELAYA. Rama.

This survey was aided in many ways by the wholehearted cooperation and enthusiastic support and assistance of many Nicaraguan government officials, public health officers, physicians and citizens, and of United States army and navy personnel, and engineers of the Inter-American Highway. Particular thanks are due Colonel Luis M. DeBayle, Director of Health Activities for Nicaragua, and Dr. Leonard S. Rosenfeld, Director for the Division of Health and Sanitation of the Institute of Inter-American Affairs.

Acknowledgment is gratefully made to Colonel W. H. W. Komp of the United States Public Health Service for determining and verifying determinations of many of the mosquitoes, and to the several members of the Division of Insect Identifications of the United States Bureau of Entomology and Plant Quarantine, particularly to Dr. C. F. W. Muesebeck, Dr. Alan Stone, and Dr. H. E. Ewing, for verifying and making determinations of most of the insects and arachnids.

SUMMARY

The results of a nine-weeks survey in selected localities of the Republic of Nicaragua are presented as a contribution to a fund of information upon which might be based the formulation of programs for the control and eradication of arthropod-borne diseases. A list is given of 124 species which are distributed among the Culicidae (38), Muscoidea (15), Tabanidae (14), Mallophaga and Anoplura (8), Acarina (5), and several other groups (44), with geographical distribution records and life history notes. The probable vectors and the transmission of prevailing arthropod-borne diseases are discussed. Malaria, the dysenteries, typhoid, myiasis due to *Dermatobia hominis*, and perhaps filariasis, are common. Suitable vectors are numerous, widely distributed, and uncontrolled.

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ACTINOMYCOSIS IN A *HYDROCHOERUS ISTHMIUS* GOLDMAN (ISTHMIAN CAPYBARA OR PONCHO)¹

CARLOS CALERO M.², AND PEDRO ORTIZ O.³

The purpose of this paper is to report a case of Actinomycosis in a captive *Hydrochoerus isthmus* Goldman (Isthmian Capybara or Poncho) at the Gorgas Memorial Laboratory, with primary apparent localization of the infection in a tumor-mass under the liver.

Report of the case: male animal, nine years of age, of the species *H. isthmus*, was taken from the Darien Province, Republic of Panama, to the Gorgas Memorial Laboratory on September 25, 1939, where it was kept in captivity, with other animals, for experimentation. During this time it had no disease, and its food was prepared and controlled by the caretaker of the laboratory. The animal ate this food well.

His food consisted of a mixture of apples, bananas, melon, corn, sugar cane, bread and cooked rice. In addition, it ate grass in the yard of the laboratory as well in the cage where he was kept at night.

The animal grew and was in good health in the laboratory for six years and eight months; at the end of this time it was noted that the animal presented anorexia and started to lose weight and was lame in the right fore leg. In view of a possible tuberculous infection the animal was killed to determine the exact etiology of its disease.

Autopsy revealed the following *macroscopic findings*.⁴

Male Poncho, Lab. No. 15, admitted to the animal house of the Gorgas Memorial Laboratory on September 25, 1939. At that time it weighed 36 lbs. and was considered as a half-grown animal since the average weight of fifteen adult Capybaras was 65 lbs.

The animal was killed with an anesthetic on August 2, 1946. Weight at time of death was 43 lbs.

External Appearance: Emaciation; the skin over the left shoulder was almost hairless and the skin was parchment-like, hard and wrinkled. The sole of the right fore-foot was swollen.

Head and neck: The skull was saved for a museum specimen and was not opened. The brain, therefore, was not examined. Eyes, tongue and neck structures were all normal.

Thorax: The pleura, visceral and parietal, of both lungs showed many milky milk-white plaques. The left lung revealed many more than the right one.

¹ The authors are much indebted to Dr. Herbert C. Clark, Director of the Gorgas Memorial Laboratory, for the excellent facilities provided for this work and his valuable suggestions.

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³ From the Gorgas Memorial Laboratory.

⁴ Gross notes by Dr. Herbert C. Clark, Pathologist and Director of the Gorgas Memorial Laboratory.

Both lungs were inflated but the base of the right lung contained two white nodules that were snow white on section and each was about 1 cm. in diameter. The thoracic lymph nodes were slightly enlarged and the sets in both axillary spaces were greatly enlarged.

Heart and pericardium: The mitral valve was thin and transparent except for the large leaflet which contained two thin acute vegetations about 3 mm. in diameter. The myocardium and pericardium were normal. The aorta and its large branches were normal. Oesophagus was normal. Thymus gland was normal.

Abdomen: The peritoneal fluid was as clear as water and about 6 ounces in amount. No flukes or other parasites were found.

Stomach and intestines: Normal. No parasites.

Liver: The liver, gallbladder and ducts were normal. Attached to the under surface and pushing upward into the Spigelian lobe was a snow-white mass as large as an English walnut and on section it contained a yellow-white, dry cheesy material. The other epigastric glands were enlarged but not cheesy in character.

Spleen: Normal consistency, color and size. No adhesions.

Kidneys and Adrenals: Normal.

G. U. system: All organs normal.

The *microscopic examination* of the tumor located under the skin of the right fore-foot showed actinobacilliform masses, resembling clubs, in the periphery of what appeared to be a Ray-fungus, as reported by one of us in cases of Pulmonary Actinomycosis (1) and Madura Foot (2). Around the border of the masses there was intense leucocytic infiltration of neutrophils and especially of polynuclear eosinophils, with foam cells, limited peripherally by a distinct capsule of adult connective tissue. In the center of the granuloma and within the space limited by the clubs were many bodies which by their form and especial distribution we suspected as being spores.

Lobulated nodules were observed, with peri-capsular adult connective tissue. In some of the lobules thus formed, we noted foam cells and marked polymorphonuclear eosinophil infiltration.

In the center of other lobules and forming the center of another granuloma (fig. 1), there were adult giant cells, some with and others without phagocytosed actinobacilliform masses.

Some granulomas presented in the center, round, spore-like bodies and branching filaments coming out in a mass of foam cells (fig. 2), and eosinophil infiltration surrounded by a capsule of fibrous connective tissue.

Not all the granulomas were of the type III described as adult (3). In fact, in the same tumor-mass, there were granulomas with young giant cells, described as type I (4), generally surrounded by young connective tissue. None of these young giant cells contained clubs.

The round tumor located under the liver was surrounded by a thick capsule of adult connective tissue with eosinophil infiltration. The nucleus of the nodule was formed by necrotic tissue in which we could distinguish thick fibrous tissue

cords with irregular distribution; irregular bodies that appeared like degenerated clubs according to the findings previously reported, but in itself not sufficient to permit a definite diagnosis; also, a large infiltration by polymorphonuclear cosino-

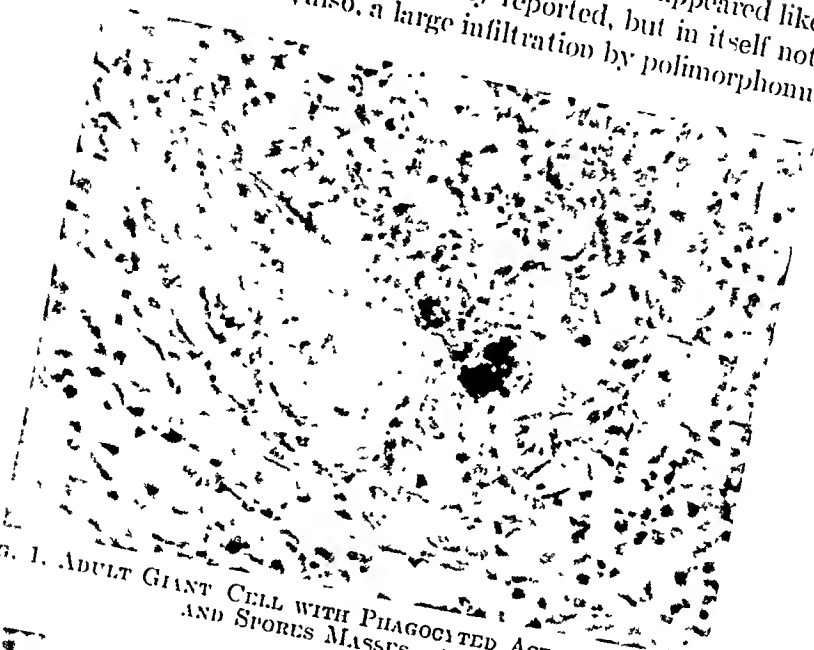


FIG. 1. ADULT GIANT CELL WITH PHAGOCYTED ACTINOBACILLIFORM AND SPORES MASSES. $\times 200$

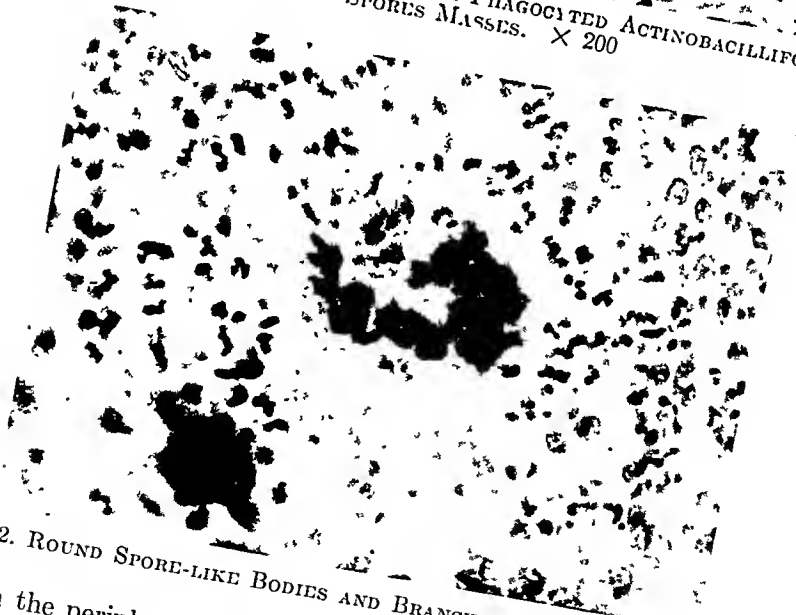


FIG. 2. ROUND SPORE-LIKE BODIES AND BRANCHING FILAMENTS. $\times 500$

philic cells in the periphery of the tumor. There was no invasion of the liver tissue.

The tumors located in the base of the right lung were encapsulated by a thick layer of adult fibrous connective tissue, at the periphery of which there was a layer of young connective tissue with marked infiltration by round cells and especially by eosinophil cells. The nucleus of each tumor-mass was formed by

necrotic tissue in which it was not possible to establish any cellular differentiation. There was congestion of the lung vessels around the tumor.

We did not see any pathology in the lymph nodes and spleen with the exception of marked eosinophil infiltration.

The examination of a piece of striated muscle fibres showed a large Sarcocystis of about 0.4 cms., with the typical microscopic arrangement reported (5), as was identified by Dr. Herbert C. Clark of this laboratory.

Animal Inoculation: With the findings previously reported several portions of the tumor-mass located under the skin of the right fore-foot and liver (which had been preserved for 10 days in a 10% formalin solution) were washed in running water to be cultured and inoculated, in spite of the fact that negative results have been reported in similar conditions. At the end of this period, the

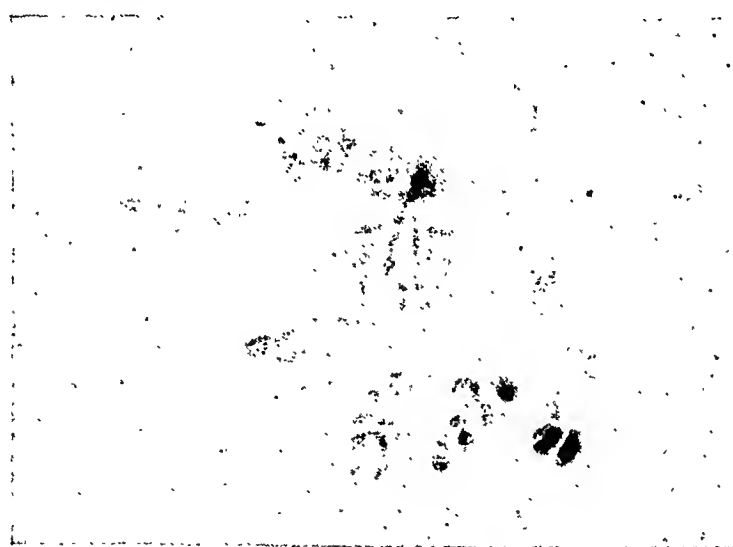


FIG. 3. GRAM NEGATIVE ACTINOBACILLIFORM BODIES. $\times 750$

tissues were triturated in normal saline solution and 1 c.c. of fine suspension was inoculated subcutaneously in a rabbit, a mouse and a guinea pig; and equal doses were injected intraperitoneally in a mouse and a guinea pig.

The investigation, as might have been expected, was negative.

Culture: A portion of the triturated tumor tissue located under the skin of the right fore-foot was inoculated in Sabouraud's prove medium and incubated at room temperature (between 26 and 30 degrees C.), aerobically and anaerobically. Daily observations were made of the plates for 28 days. The investigation was negative.

Staining characteristics: Examination of the organism encountered regarding its staining properties with the colorants commonly used in pathology (hematoxylin and eosin) showed (fig. 1), that the central area of what resembled Ray-fungus, consisting of the round sporocytic bodies, stained deeply with hematoxylin; and that the peripheral portion, formed of the actinobacilliform rods, had a marked affinity for eosin.

It was also demonstrated that the organism was (fig. 3) Gram-negative (6) and acid-fast.

COMMENTS

Considering the time the animal was in captivity in the laboratory and the site of localization of the tumor-mass with actinobacilliform bodies we think that the animal acquired the infection during captivity.

Autopsy of the animal, performed twenty minutes after it was killed, showed a tumor-mass under the skin of the right fore-foot in which we found microscopically an organism like Ray-fungus, with actinobacilliform bodies, peripherally located, which were Gram-negative, acid-fast and had marked affinity for eosin; and by round bodies, centrally located, with marked affinity for hematoxylin.

The appearance of the organism found (fig. 2) gave the impression that the disease of the *H. isthmus* (Capybara) was due to a species of Actinomycosis which we consider, according to its morphology and staining properties, in the family Streptomycetaceae of Waksman and Henrici (7). We were unable to classify the organism more specifically because of negative animal inoculation and culture of the tumor-tissue, which had been preserved for 10 days in a 10% formalin solution.

It is important to call attention to the polymorphonuclear eosinophilic infiltration found in each of the slides prepared of the various tumor-masses found at autopsy. And finally that the histopathologic findings in the tumor masses located under the liver and in the base of the right lung were so similar that it was impossible to distinguish one from the other and that in neither was there found any pathogenic actinobacilliform organism.

SUMMARY

A case of Actinomycosis is reported in a captive *Hydrochocrus isthmus* Goldman (Isthmian Capybara or Poncho), at the Gorgas Memorial Laboratory.

Staining characteristics showed that the actinobacilliform rods had a marked affinity for eosin and were Gram-negative and acid-fast; and that the central area of what resembled Ray-fungus, consisting of the round sporocytic bodies, stained deeply with hematoxylin; giving the impression that the organism was due to a species of Actinomycosis of the family Streptomycetaceae of Waksman and Henrici (7).

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TROPICAL PHAGEDENIC ULCER (VINCENT'S ULCER)

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The recent war has greatly increased the interest in tropical phagedenic ulcer and related diseases. It is the purpose of this paper to summarize the advances which have been made in this condition in the past ten years, and to emphasize the use of penicillin in its treatment.

DEFINITION

Tropical phagedenic ulcer is a specific, acute, ulcerative skin disease with a characteristic slough containing numerous fusiform bacilli and spirochetes in its early stages. If neglected, it may develop into a chronic nonspecific ulcer which is indistinguishable from indolent ulcers resulting from other causes.

TERMINOLOGY

Most writers believe that tropical ulcer is a definite entity (69) which was first described in the middle of the last century (11). Confusion has arisen in the literature from failure to reserve the term "tropical ulcer" for one specific type of lesion. It was, for example, confused with cutaneous leishmaniasis by the authoritative Standard Nomenclature of Disease (37) and with cutaneous diphtheria by the U.S. Army Medical Department (22) and by Liebow *et al.* (38). Discrepancies of this sort have led some to recommend discarding the term (20). Wide usage however, by many authors (61, 62) demands that it be retained. The word "phagedena" (which means cancerous sore) (57) is included by many (61, 43) to distinguish this disease from other ulcers in the tropics. The term "Vincent's ulcer" may also be added as Costa (16) has suggested for further clarity. It is one of the purposes of this paper to attempt to clear up some of the semantic confusion which exists in the literature of this complex disease.

In addition to the difficulties which arise from the use of a too-inclusive term, are those which arise from the use of geographic or native names for this lesion. Although it is important to know the area in which published studies are performed, use of accepted nomenclature will prevent confusion in the literature. The following synonyms have been compiled: Annam ulcer (43), Aden ulcer (62), Chaco (Argentina) ulcer (16), Coast ulcer (Parana) (16), Cochin sore (11), Delago Sore (11), Frontier sore (13), Garigha (49), Goyana ulcer (16), Malabar ulcer (62), Mozambique ulcer (43), Naga sore (61), Natal sore (11), Phagedena tropica (37), Phagedenic ulcer of hot countries (16), Rhodesian sore (11), Tropical sloughing phagedena (61), Ulcus tropicum (61), Vincent's ulcer (16), Yemen ulcer (43), and Zambesi ulcer (11).

GEOGRAPHICAL DISTRIBUTION

Tropical phagedenic ulcer is widely distributed in the warmer countries throughout the world. As Costa (16) points out however, it occurs in subtropical and temperate climates as well as those considered as strictly "hot countries". The accompanying map (fig. 1) has been prepared to indicate the areas from which this disease has been reported. The literature was carefully reviewed but some reports may have escaped notice. The following countries are indicated:

SOUTH AND CENTRAL AMERICA

Amazonia (Stitt, 61) including Bolivia and Brazil; Argentina (Costa, 16) including Chaco and Parana (Paraguay); British Guiana (Romiti, 14); Jamaica (Clements, 11); Trinidad (Earle, 26);

AFRICA

Algeria (Brault, 27); Belgian Congo (Stitt, 61); Cameroons (Clements, 11); French Equatorial Africa (Leboeuf, 27); Gambia (Clements, 11); Gold Coast (Clements, 11); Kenya (Stitt, 61); Liberia (Clements, 11); Madagascar (Fontoy-mont and Gourdran, 27); Mozambique (Manson-Bahr, 43); Nigeria (McCulloch, 14); Nyasaland (McGill, 46); Sierra Leone (McGill, 46); Somali (Davies, 10); Sudan (Corkhill, 14); Tanganyika (Buchanan and Sanderson, 14); Tripoli (Clements, 11); Uganda (Locwenthal, 14); Zambesi (Bruce; Wolbach and Todd, 27) including Angola and Rhodesia;

ASIA

Aden (Sutton, 62); Burma (Grindlay, 32); China (Eggers, 27); Dutch Indies (Clements, 11); India including Assam, both frontiers, (Col. Med. Rep., 13) Madras (Seshadrinathan, 54) and Orissa (Pattanayak, 50); Indo-China (Regnault, 27); Java (Prowazek, 27); Malaya (Stitt, 61); Melanesia (James, 61) including New Guinea, New Britain (Manson-Bahr, 43) and the Solomons; Palestine (Gill, 31); Persia (Carr, 27); Philippine Islands (Corpus, 15); Siam (Mendelson, 15); Singapore (Eggers, 27); Syria (Clements, 11); Yemen (Manson-Bahr, 43); and

EUROPE

Baku, Caucasia (Clements, 11).

CLINICAL CHARACTERISTICS

The lesions of tropical phagedenic ulcer occur almost exclusively on the lower extremities. In the hundreds of cases reported by Earle, none were above the knee (26). Pattanayak states that the lower limbs are invariably involved, with occasional secondary spread to the upper limbs (50). In occasional cases the lesions are confined to the distal half of the terminal phalanges of fingers and toes with destruction of the nail beds (25, 33, 46).

As several writers point out, there are three distinct stages in the development of a tropical ulcer (46, 50). The first stage may last only 12 to 24 hours and is

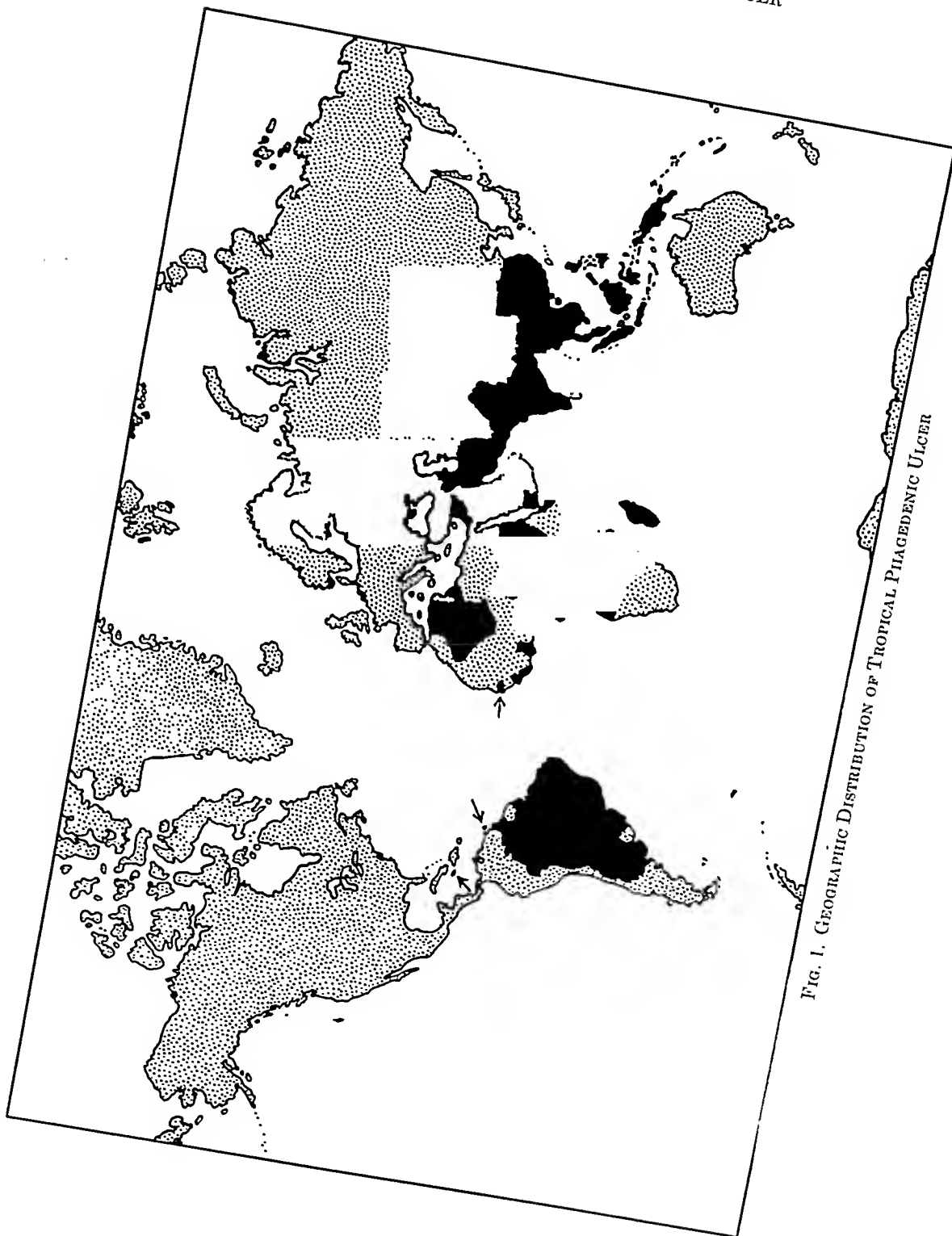


FIG. 1. GEOGRAPHIC DISTRIBUTION OF TROPICAL PHAGEDENIC ULCER

therefore rarely seen by the physician. This earliest lesion develops in over 65 per cent (11) of patients at the site of trauma. The injury usually is of a crushing or bruising nature (26) but may be only a minor laceration or abrasion. The lesion first appears as a painful, tense, swollen area, with a blunt conical swelling or a serosanguinous bleb at the site of injury. Within a few hours as astonish-



FIG. 2. TROPICAL PHAGEDENIC ULCER ON THE LATERAL ASPECT OF LEFT LOWER LEG, EARLY STAGE, UNTREATED. TAKEN NOVEMBER 27, 1944
(U. S. Army Museum & Medical Arts neg. no. A44888-1)

ingly rapid sloughing process involves the entire site, and spreads in all directions (26, 33, 43).

The second, or acute phagedenic phase, presents striking characteristics which have been described by many observers (fig. 2). The painful ulcer is usually oval or circular, and varies from two to many centimeters in diameter. It has a foul odour and is entirely filled with a yellowish to blackish, purulent, gangrenous slough which is firmly attached to the base (11, 16, 36, 43, 61, 69). The ragged overlapping edges of the ulcer assume a bluish red tint and are raised slightly above the surrounding tissue (23). The edges are often undermined for 1 to 2 centimeters by the subcutaneous spread of the sloughing process (34,

54). The base of the lesion is irregular, and may go no deeper than the fascia (11), although in some countries it has been reported to involve tendons (36, 43), and even bone (43, 53), with disastrous results. During this stage the ulcer is loaded with fusiform bacilli and spirochetes (11, 16, 26, 33, 34, 50, 54, 61) and has the characteristic histopathologic features described below.

The third or chronic stage gradually develops over a period of weeks to months if the ulcer is neglected or inadequately treated during the acute phagedenic phase. In such lesions of long duration, the characteristic slough is no longer present, except perhaps for a small amount at the mouths of small sinuses, and as a result the odour is much less offensive (11). All pain is gone and even vigorous manipulation is tolerated (23). The sides of the ulcer become very hard and assume a rolled appearance. The base, which is also very hard, becomes even and filled with granulations which do not bleed easily. Scrapings may or may not reveal fusiform bacilli and spirochetes.

In this last stage, the appearance and histopathology differ little from chronic indolent ulcers due to many other causes. Bacteriologic studies are also of little help in diagnosis, because the fusospirochetes have frequently disappeared (26, 50). Studies based primarily on lesions seen in this nonspecific stage have been an important cause of the disagreements in the literature.

HISTOPATHOLOGIC CHARACTERISTICS

The characteristic histopathology is seen only in sections taken from an actively phagedenic part of the ulcer. The central slough or "pseudomembrane" is a structureless mass of tissue which has undergone coagulation necrosis, and is interspersed with degenerating leukocytes, strands of fibrin, and an enormous number of fusiform bacilli and spirochetes, with occasional other bacteria. A dense layer of fusiform bacilli forms a barrier between the outer slough and the deeper vascular granulation tissue. The granulation tissue extends to the fascia and contains a great number of polymorphonuclear leukocytes, particularly at the upper edge (11, 13, 61).

Some earlier authors who examined sections from indolent portions, or from chronic ulcers, failed to find any such characteristic picture.

ETIOLOGY

Tropical phagedenic ulcer so rarely affects Europeans or well-to-do natives, that it has been called a "class" or "poor man's disease" (29, 36, 46). Livingston (39), Liebow (38), and the author very rarely observed it among many thousands of American troops stationed in areas where the disease was endemic among the natives and the Chinese soldiers. This is particularly significant, because many cases of specific infections such as cutaneous diphtheria and cutaneous leishmaniasis occurred frequently in American troops. Since the opportunity for infection with tropical phagedenic ulcer among these troops was apparently as great as with diphtheria or leishmania, one can only conclude that the problem is not simply one of infection, but rather of some change in the host which allows the lesion to develop.

There is evidence however, that an infectious agent is at least partially re-

sponsible for this disease. Epidemics and other suggestive epidemiologic data have been repeatedly observed (15, 36, 43, 50). Webb however, feels from his experience in southern China, that even though the ulcers appeared almost like an epidemic among the badly fed and disease ridden Chinese troops, there was no evidence of infection from one to another (70). Although Dostrovsky and Sagher (23), Mohanty (47), and Liebow et al. (38) (who were apparently dealing only with cutaneous diphtheria) did not find fusospirochetes in their material; Strong (61), Manson-Bahr (43), Sutton (62), Clements (11), Costa (16), Panja (13), Jarvis (36), Forbes Brown (29), Gunther (33), Marsh and Wilson (44), Corpus (15), Eggers (27), Pattanayak (50), Seshadrinathan (54), Webb (70), Earle (26), Hamm and Ouary (34), and others have consistently confirmed von Prowazek's original observation of the overwhelming preponderance of Vincent's organisms in tropical phagedenic ulcer. Fusospirochetes are so ubiquitous and yet so difficult to culture in an uncontaminated form, that the various successful inoculation experiments (11, 13, 61) cannot as yet be considered as having completely solved the question of the nature of the invading organism. Particular caution should be exercised in ascribing etiologic importance to any bacterial organism in the light of Black's evidence (8) that Vincent infections of the mouth are due to the virus of herpes simplex. Stiegman and MacNair Scott (59) however, were unable to obtain the virus of herpes simplex from any of 18 cases of Vincent's angina and concluded that the fusospirochetes were of etiologic importance. In addition, Panja (13) was unable to pass the infectious material with which he could reproduce tropical ulcers through a Chamberland L3 or L5 filter.

In addition to the fusospirochetes, numerous investigators have studied the conditions which predispose the host to invasion by these organisms. Certain factors seem quite important, but as yet there is not complete agreement on the conditions which are of primary importance. The factors which have been incriminated at various times include: dietary deficiencies, dietary excesses, debility from disease, trauma, climate, alcoholism, vascular stagnation, tissue inertia, parathyroid deficiency, and various combinations of these (14, 15, 33, 44, 47, 50).

Of this group, dietary deficiency has received the most attention. While some believe that general malnutrition is essential (14, 16, 44, 54) there are a few reports of the disease in healthy robust individuals (43, 50). Inadequate amounts of dietary protein (11, 14, 29, 46) or of dietary fat (11) are also commonly encountered in natives with tropical ulcers. Although several observers (14, 29, 46, 61) agree with Loewenthal (40) that calcium deficiency is important, Earle (26), and particularly Clements (11), have shown that the disease can occur in natives with normal blood calcium levels, who eat a great excess of calcium (as powdered lime with betel nut). Many writers agree that vitamin deficiency is the really critical factor (2, 14, 36, 46). Others have carried their investigations farther in an attempt to determine the particular essential vitamin. Although Charters (10) feels that vitamin A is important, Corkhill (14) does not agree. One of the most convincing pieces of work indicating that a vitamin B deficiency,

is often confused with cutaneous diphtheria, although Cameron and Muir (9) and Gill (30) who have had a wide experience with both of these lesions do not consider them identical.

Cutaneous diphtheria is also usually multiple, occurring on both upper and lower extremities (9), although in the author's series of eighty-two cases, the majority were on the legs. These ulcers, which occur in Europeans, are usually circular or oval and have punched out, clear cut borders. The margins are hard and thickened with a bluish colored, rolled appearance (9, 18, 51). The base may be relatively even and clean, or may in the early stages, have a blackish, tough, leathery adherent membrane covering it. As Livingood (39) and others have demonstrated, diphtheria may also occur as a secondary infection in other skin diseases such as eczema, with resulting indolence of lesions and toxic symptoms. Virulent *Corynebacterium diphtheriae* can usually be isolated with careful technic (21). Fusospirochetes are not found.

Ecthyma due to pyogenic streptococci and staphylococci, is extremely common among all races in the tropics. It is usually the result of an infection of an insect bite, an abrasion, a laceration, scabies (1), or other form of cutaneous trauma. It differs little from that seen in the temperate climates except perhaps in being more extensive and a little slower to heal. It is a superficial ulcer without undermined edges, and contains either frank pus or a pyogenic, yellowish brown, easily removed, crust.

Cutaneous leishmaniasis (Oriental sore, Bagdad boil) strongly resembles cutaneous diphtheria (9). It occurs in Europeans as well as natives, is usually multiple, and appears on the arms, legs, or face. The lesions vary from papules to large ulcers with punched out sides and a lip like border. The central ulcer is covered by a thick, rough crust which is difficult to remove. Leishmania tropica are obtained from scrapings (4).

Syphilitic gummata occur in all races. Their punched out character, along with other classical features distinguish them from tropical phagedenic ulcers (11). Neither *Treponema pallida* nor fusospirochetes are found on routine microscopic examination. The possibility of tropical ulcers occurring in a person with an unrelated positive serologic test for syphilis should be kept in mind.

Other conditions which cause ulcers of the skin which might be confused with tropical ulcers are: varicose ulcers (43), decubitus ulcers, primary or tertiary yaws (29, 43), leprosy (61), amocbiasis cutis (43), blastomycosis (61), sporotrichosis (61), granuloma inguinale (61), pyoderma ulcerosum tropicalum (if proven to be distinct from other ecthymatous lesions) (1), and even histoplasmosis (3).

In addition to tropical phagedenic ulcer of the skin, Gauthier (33) describes a lesion occurring in the mouth with the same appearance, bacteriology, and response to therapy as tropical ulcer, which he calls "New Guinea mouth disease." This appears however, to be the same as noma (gangrenous stomatitis). There is much in the appearance of the debilitated patient and the gangrenous sloughing lesion with many fusospirochetes (6) that is similar in the two diseases.

As Strickler (60), and Benedict (5) point out, Vincent's disease of the skin,

occurs as a great rarity in the United States. Although their two cases were indolent, foul smelling, and contained many fusospirochetes, they did not produce phagedenic lesions.

TREATMENT

A wide variety of therapy has been proposed for tropical ulcers. These are summarized by Stitt (61), Manson-Bahr (43), Clements (11), and others. Correction of dietary deficiencies and treatment of systemic diseases is of course good general therapy, but does not in itself cure the lesions when they have once developed. Treatment directed to the ulcers should be divided into two classes; measures which will clear up the phagedenic process, and measures which will more quickly heal a clean ulcer or a chronic indolent ulcer.

Because of the many treatments which have been recommended in the past for stopping the phagedenic extension and rapidly cleaning up the sloughing ulcer, one is inclined to believe that the ideal treatment was not among them. Most agree that general medical principles apply here; *i.e.* with local antiseptics, debridement, and rest, the ulcer will eventually be cleaned up. The search has been for the antiseptic that would be rapidly effective in all cases. Of the recent ones, copper sulphate as recommended by Gunther (33), has been popular. The ointments "bipp" and "zipp" (bismuth or zinc oxide with iodoform and petrolatum) have been found to be of some help (44). The value of sulfonamides as proposed by Earle (24) has also found recent confirmation (23). The spirocheticidal metals have probably received the most thorough trial of any group of drugs. They are however, apparently of little value (11, 26, 70), although some of the earlier writers and Feinman (28) thought them useful. It is interesting to note the ineffectiveness of arsenicals and bismuth in Vincent's lesions of the mouth as well as in tropical phagedenic ulcer. As Ludwick (41) and others, including the author have noted, acute Vincent's infection of the oral cavity may develop in a patient during a course of intensive systemic arsenical (Mapharsen) and bismuth therapy. The parallelism between therapy in Vincent's infections of the mucous membranes and tropical ulcers is further shown in the section on penicillin.

After an acute ulcer has been freed of the phagedenic process it will fill with granulation tissue and epithelize if protected from trauma and further infection. Skin grafting will greatly speed this process (56). At the base of the old chronic ulcers however, the granulations are fibrosed and do not bleed easily. Before grafting such lesions it is usually necessary to first remove this hard tissue by vigorous curettage or complete excision. Prophylactic use of penicillin at this time should prevent relapses or failures of the grafts to take. ACS (anti-reticular cytotoxic serum) was recommended by Mashkilleison in chronic resistant ulcers (45). Local ice therapy has also been suggested (65).

PENICILLIN IN VINCENT'S INFECTIONS

Shortly after the first demonstration of the spirocheticidal action of penicillin by Mahoney, Arnold, and Harris (42), the effectiveness of the drug against the

leptospira of Weil's Disease was reported by Heilman and Herrell (35). This was followed within three months by the first report of the use of penicillin in oral Vincent's infections by Denny, Shallenberger, and Pyle (19). The early encouraging results were quickly substantiated by a number of investigators who reported clinical improvement in Vincent's infections including both angina and gingivitis. Their results demonstrated the disappearance of the organisms within 12-72 hours (48, 55, 58), subjective improvement in 24 hours (55, 58) and rapid clinical cures in all cases treated either locally (12, 58) or by intramuscular (48, 55, 58, 63) administration of the drug.

As pointed out, the clinical similarity between noma and tropical phagedenic ulcer is great. The author's favorable experience with noma in Okinawan children treated with penicillin was confirmed by Vaizey in Ethiopia, where the disease formerly carried an 80 per cent mortality (67).

PENICILLIN IN TROPICAL PHAGEDENIC ULCER

Although absolute proof of the etiologic role of fusospirochetes in tropical ulcers, as in Vincent's angina or gingivitis is not available, the fact that they are the predominant organisms present, would lead one to believe that a therapy effective in Vincent's lesions of the mucous membrane would probably be effective in such lesions of the skin. The U. S. Naval Handbook of Tropical Diseases states that penicillin has been found satisfactory in the treatment of tropical ulcers (64). Hamm and Quary (34) report 18 cases of tropical phagedenic ulcer treated with local applications of penicillin. Their results were so good that they concluded that penicillin is the most efficacious treatment in this disease. Webb (70) reported ten cases treated with penicillin with excellent results, and stated that the thick base of tough scar tissue which makes the healing of ulcers so slow, did not form.

CASE REPORT

The following case demonstrates the rapid effectiveness of penicillin given intramuscularly in clearing the phagedenic phase of the disease, leaving a clean lesion which can heal naturally or be a suitable base for early successful skin grafting. It is also further proof of Marsh and Wilson's statement (44) that "even if the associated diseases are neglected, the ulcer will heal with proper treatment".

Although a number of additional patients were observed and treated for tropical phagedenic ulcer, the records of all but the following patient were lost in military movements.

A 24 year old Chinese soldier was admitted to an American army hospital in Assam, India, November 26, 1944 complaining of a painful ulcer of his leg. Through an interpreter it was learned that the lesion had been present for about seven days, during which time no treatment but a dry dressing had been applied. He had never had a similar lesion. The soldier had been stationed in the jungles on the "Ledo Road" near the India-Burma border. He had had two episodes of fever (probably malaria) and one period of dysentery during the preceding year.

Physical examination revealed a thin Chinese male who appeared undernourished and chronically ill. He had no fever. In addition to the leg lesion, the only other physical finding of note was a barely palpable spleen. On the lateral aspect of his left leg, just above the ankle, was a large, foul smelling, deep, rounded ulcer, the diameter of which was almost equal to the width of his leg at that level (fig. 2). There was very little edema around the ulcer. Its sides



FIG. 3. TROPICAL PHAGEDENIC ULCER, AFTER TWO DAYS OF PENICILLIN THERAPY. TAKEN
NOVEMBER 30, 1944
(U. S. Army Museum & Medical Arts neg. no. A44914-2)

were irregular and deeply undermined, producing small flaps of overhanging skin. The ulcer was filled with a semisolid blackish purulent slough. The contour of several tendons could be seen in the depths of the ulcer, and in one corner of it maggot was detected. The regional inguinal lymph nodes were slightly larger than those of the opposite side but were not tender. Roentgen examination of the lower left leg did not disclose any bone changes. Roentgen examination of the chest was also negative.

Laboratory examinations revealed a large number of fusiform bacilli and spirochetes in a direct smear from the ulcer. Examinations of deep scrapings for leishmania were negative. Cultures on blood agar and on Loeffler's media under aerobic conditions disclosed only non-hemolytic staphylococci. On examination of his peripheral blood it was found that he had 3.2 million erythrocytes, with 7.5 grams of hemoglobin (Sahli). The total white blood cell



FIG. 4. TROPICAL PHAGEDENIC ULCER, ONE WEEK AFTER PENICILLIN THERAPY. TAKEN
DECEMBER 5, 1944
(U. S. Army Museum & Medical Arts neg. no. A44932-1)

count was 6,200 with 62 per cent polymorphonuclears, 26 per cent lymphocytes, and 12 per cent eosinophils. No parasites were found in the peripheral blood smears. The serologic test for syphilis (Kahn) and complete urinalysis were negative. Ova of both *ascaris lumbricoides* and *ancylostoma duodenale* were found in the stools.

To evaluate fully penicillin therapy, no other treatment was given for the first week. The patient was ambulatory in the hospital area. Two days after admission, on November 28, 1944, penicillin sodium, 20,000 units was admin-

istered intramuscularly every three hours. This was continued until November 30, for a total of eighteen doses, 360,000 units. At this time, smears for fusospirochetes were negative and the improvement was so dramatic (fig. 3) that the penicillin was discontinued. Observation one week later, without further systemic or local treatment other than an adequate diet, showed continued healing (fig. 4). He was then started on other medication which included:



FIG. 5. HEALED TROPICAL PHAGEDENIC ULCER, FINAL RESULT AFTER PENICILLIN THERAPY.
TAKEN MARCH 15, 1945
(U. S. Army Museum & Medical Arts neg. no. A45354-1)

multi-vitamin supplements, vermifuges, and ferrous sulphate. He gradually gained weight and vigour, and his blood count returned to normal. The skin lesion epithelized over the healthy granulation tissue without difficulty. It was possible to reexamine the patient ten weeks later, at which time a thin but satisfactory scar was noted (fig. 5).

SUMMARY

1. Tropical phagedenic ulcer is a distinct entity with a characteristic appearance. It occurs on the lower extremity as a large rounded ulcer with

undermined edges, and is filled with a wet gangrenous slough, in which fusiform bacilli and spirochetes predominate in the bacterial flora.

2. If inadequately treated, the lesion may persist as a chronic ulcer with a hard fibrosed base and border. In this stage there are no longer any bacteriologic or histopathologic features which distinguish it from other indolent ulcers. It is important, from the standpoint of both prognosis and therapy, to differentiate between the acute phagedenic ulcer and the chronic non-specific ulcer.

3. Other diseases to be considered in differential diagnosis are: desert sore, cutaneous diphtheria, pyogenic ecthyma, cutaneous leishmaniasis, syphilitic gummata, and other rarer ulcerative lesions.

4. The geographic distribution of the disease is presented.

5. The etiology is discussed. The preponderance of evidence to date indicates that the ulcers are caused by Vincent's fusospirochetes in a host who has become susceptible to infection as a result of a vitamin B deficiency, and who is possibly further debilitated by chronic disease and general malnutrition.

6. The successful treatment of Vincent's infections of the mouth: gingivitis, angina, and noma with penicillin is recorded. The superb results of other investigators and of the author in treating the active phase of tropical phagedenic ulcers with penicillin indicates that it will probably become the treatment of choice. The chronic stage should be treated surgically.

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EXPERIMENTAL STUDIES WITH *PASTEURELLA TULARENSIS* IN FISH*

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During March and April, 1946, an epizootic of tularemia occurred (1) in aquatic mammals (muskrat and beaver) near the Horicon Marsh, Dodge County, Wisconsin. The State Conservation Department raised the question whether the fish in the marsh might become infected with *Pasteurella tularensis* and serve as a potential reservoir of the disease and transmit the disease to anglers. Miller (2) reported that catfish had been involved in infecting persons with tularemia. Whether the catfish actually infected the persons or made the puncture (fin prick) by which the bacteria entered was not definitely determined. In 1940 the Bureau of Biological Survey issued a release (3) on the potential danger of catfish transmitting tularemia.

Several investigators have reported the isolation of *P. tularensis* from river water. Karpoff and Antonoff (4) in Russia first suggested that water may be naturally contaminated with *P. tularensis*. A waterborne epidemic of tularemia was reported from Turkey by Gotschlich and Berkin (5). Parker, *et al.* (6) isolated the organism from three Montana streams. Jellison, *et al.* (7), in a study of spontaneous tularemia in beaver, found both water and mud of streams contaminated. They also suggested the possibility of beaver acquiring the disease from contaminated water. Parker, *et al.* (8) found tularemia in beaver and muskrats in association with *P. tularensis* contaminated water and mud.

Green (9), Scott (10), and Hammersland and Joneschild (11) reported the natural occurrence of tularemia in the beaver as probably due to a waterborne infection. Burroughs, *et al.* (12) presented a list of all known vertebrates naturally infected with tularemia but no species of fish were mentioned.

Since *P. tularensis* may survive for a long period of time in natural waters, an ideal method would appear to be provided by which fish may be continually exposed to the disease. The purpose of this paper is to report our findings on the exposure of six species of fish with a highly virulent strain of *P. tularensis*.

The species of fish used in these studies were the black bullhead, *Ameiurus m. melas* (Rafinesque), black crappie, *Pomoxis nigro-maculatus* (Le Sueur), large mouth bass, *Huro salmoides* (Lacepede), northern pike, *Esox lucius* Linnaeus, yellow perch, *Perca flavescens* (Mitchill), and rainbow trout, *Salmo gairdnerii irideus* Gibbons. Ten fish of each species were injected intraperitoneally with 1 ml of a 48 hour culture of *P. tularensis*. This suspension was standardized by using an Evelyn Colorimeter with a 660 filter giving a galvanometer reading of 60 per cent transmission of light. At 24 hour intervals one fish of each species was sacrificed and examined thoroughly for lesions. At these times an attempt

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and the Conservation Director.

was made to isolate the organism. The liver, spleen, and kidney were removed and ground with a sterile mortar and pestle. One ml of this emulsion from each fish was injected intraperitoneally into separate guinea pigs. After 14 days each guinea pig was sacrificed and examined minutely for lesions of tularemia. All of the organs were routinely cultured for *P. tularensis*.

The strain of *P. tularensis* used in this study was originally isolated in 1938 from a snowshoe hare in Vavenby, British Columbia. This strain was furnished through the courtesy of Dr. R. R. Parker, Rocky Mountain Laboratory, Hamilton, Montana. The organism was cultured on cystine blood agar.

The fish were maintained in large rectangular tanks with a capacity of approximately 50 gallons. In the case of trout, running water was allowed to overflow into a chlorine trap. Five fish of each species were kept in single tanks with the exception of the northern pike which were in separate containers.

TABLE 1

Results on the intraperitoneal injections of six species of fish with *Pasteurella tularensis*

NAME OF FISH	NUMBER OF FISH INJECTED WITH PASTEURILLA TULARENSIS INTRAPERITONEALLY	CONTROL FISH INJECTED WITH SALINE	CONTROL FISH NOT INJECTED	NUMBER OF GUINEA PIGS INJECTED WITH ORGANS FROM FISH	NUMBER OF GUINEA PIGS INJECTED WITH EMULSION OF CONTROL FISH ORGANS	RESULTS OF ISOLATION	LESIONS	AGGLUTINATION ON FISH AND GUINEA PIG SERA	CONTROL GUINEA PIGS INOCULATED WITH PASTEURILLA TULARENSIS
Bullhead....	10	5	5	10	2	—	—	—	1
Crappie.....	10	5	5	10	2	—	—	—	1
Perch.....	10	5	5	10	2	—	—	—	1
Bass.....	10	5	5	10	2	—	—	—	1
Pike.....	10	5	5	10	2	—	—	—	1
Rainbow....	10	5	5	10	2	—	—	—	1
Total.....	60	30	30	60	12				6

Ten control fish were kept for each species; five of each group were injected intraperitoneally with 1 ml of sterile physiological saline and five were unmolested. The organs (kidney, liver, spleen) of the control fish by species were pooled, emulsified and 2 ml injected intraperitoneally into one group of control guinea pigs. Another group of 6 control guinea pigs were injected intraperitoneally with .05 ml of a 48 hour culture of *P. tularensis*. In a supplementary experiment 5 bullheads were fed one gram each of infected guinea pigs liver.

Table 1 summarizes the results of the experiment. In every case all of the fish proved negative when cultured for *P. tularensis*. With the exception of the inoculated control guinea pigs, no lesions were found in any of the guinea pigs. The fish likewise showed no lesions. All of the control guinea pigs injected with *P. tularensis* died within 6 to 8 days. Negative reactions were recorded for the agglutination tests on the sera of the inoculated fish and test guinea pigs. These negative findings indicate that certain species of fish do not become infected with tularemia. These findings suggest also that certain fish do not serve as a re-

servoir of the infection. However, there would appear to be danger that man may contract tularemia from fish caught in *P. tularensis* contaminated water, either by direct contact or wounds produced by fin pricks.

SUMMARY AND CONCLUSIONS

Six species of fish, black bullhead (*Ameiurus melas*), black crappie (*Pomoxis nigro-maculatus*), large mouth bass (*Huro salmoides*), northern pike (*Esox lucius*), yellow perch (*Perca flavescens*), and rainbow trout (*Salmo gairdnerii irideus*) was injected intraperitoneally with 1 ml of a 48 hour culture of *Pasteurella tularensis*. All of the fish were negative from 1 to 10 days after inoculation as determined by guinea pig inoculation, serum agglutination tests, and attempts at direct cultural isolation of the organism. Bullheads fed infected liver also remained negative. These negative findings indicate that certain fish are not susceptible to tularemia and, consequently, would not represent a reservoir of the disease and a public health menace.

ACKNOWLEDGMENTS

The writer wishes to thank the State Conservation Department for furnishing the fish used in these experiments and for providing some of the facilities necessary to carry on this work.

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THE FATE OF RADIOACTIVE TARTAR EMETIC ADMINISTERED TO HUMAN SUBJECTS

I. BLOOD CONCENTRATION AND EXCRETION FOLLOWING SINGLE AND MULTIPLE INTRAVENOUS INJECTIONS¹

F. C. BARTTER, D. B. COWIE, HARRY MOST,² A. T. NESS, AND SCOTT FORBUSH

In spite of the prominent place of antimonials, and in particular of tartar emetic, in therapy (1, 2, 3) there is but scant information in the literature as to the immediate and eventual metabolic fate of this drug. One of the factors contributing to this paucity of data lies in the difficulty of application to animal tissue of a suitable microanalytical method for the determination of antimony. This objection has been largely overcome by the use of the element in radioactive form. The present report deals with the results of administration of single doses of tartar emetic to human volunteer subjects, with reference to the rate of excretion, and the levels found in the blood. In addition, the excretion and early blood levels are given for one patient who received a full therapeutic course of radioactive tartar emetic.

METHODS AND PROCEDURE

Antimony metal was bombarded with deuterons in the cyclotron of the Department of Terrestrial Magnetism, at the Carnegie Institution of Washington, to produce radioactive isotopes. In this process, the naturally occurring isotopes, Sb.¹²³ and Sb.¹²¹ are transformed into Sb.¹²⁴ and Sb.¹²², respectively. The former has a half life of 60 days, the latter of 2.8 days. Both give rise to a high energy gamma ray on disintegration, a property which greatly facilitates detection, even in the presence of organic matter. Impurities of the target were eliminated, carrier added, and the antimony synthesized into tartar emetic in the Chemistry Laboratory of the National Institute of Health. Radioactivity of standards, as well as that of specimens for analysis, was determined in the Carnegie Institution by means of Geiger counters and scaling circuits.

Eight volunteers were selected from among the ambulatory, asymptomatic patients at Moore General Hospital. All 8 subjects were under observation for schistosomiasis japonica; and all but one had undergone previous antimony treatment for proved or suspected schistosomiasis. All were normal to physical examination, and all had normal kidney function as judged by routine laboratory tests. Pertinent data with regard to these patients are listed in Table 1.

Each of the first 7 patients was given a single dose of tartar emetic intravenously. The drug was previously made up into a 1.5 percent solution in distilled water and sealed in ampoules. Injection lasted from 5 to 10 minutes, depending

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on the reaction of the patient (see below). As will be seen, this factor is of primary importance in determining the immediate metabolic fate of the drug. Patient No. 8 received a course of radioactive tartar emetic according to the following schedule: Initial dose, 18.25 mg. antimony; second dose (48 hours later) 36.5 mg. antimony; 11 succeeding doses at 48-hour intervals, 54.7 mg. antimony each.

The single doses varied from 0.253 to 1.6 milligrams of antimony per kilogram of body weight. Due to a misunderstanding between the medical and the chemical workers on the team regarding content of *antimony* and of *tartar emetic* in a solution, 2 patients received a dose approximately three times that intended, namely, 1.5 and 1.6 mg. per kilogram of body weight. The latter patient suffered stomach pain, severe cough, vomiting, and diarrhea but was well in approximately 2 hours. The former patient (1.5 mg./kg.) showed the above

TABLE 1
Pertinent data on the 8 volunteers selected for study

PATIENT NO.	AGE	DOSAGE (mg. Sb./kg.)	DAYS STUDIED	PREVIOUS ANTIMONY	INTERVAL, LAST Sb. TO PRESENT STUDY
1	24	0.253	7	293 mg. in fuadin	3½ months
2	25	0.570	7	382 mg. in fuadin	3 months
3	26	0.640	28	340 mg. in fuadin	8 months
4	20	0.642	7	none	
5	23	0.781	6	510 mg. in fuadin	3½ months
6*	22	1.502	5	623 mg. in tartar emetic	2 days
7*	24	1.600	7	213 mg. in fuadin; 150 mg. in tartar emetic	4 months 2 days
8	20	10.1 (13 doses)	45	212 mg. in fuadin; 529 mg. in tartar emetic	7 months 5 months

* Patients No. 6 and No. 7 were at the end and the midpoint, respectively, of a routine Moore General Hospital course of tartar emetic for schistosomiasis.

symptoms to a more severe degree, and in addition became disoriented for a period of 3 hours from the time of injection. He appeared to be completely recovered in 8 hours, and has shown no apparent ill effects since that time. With the exception of transient nausea during injection, without vomiting, in one patient (patient No. 1), and cough during injection in one patient (patient No. 4) the remaining patients showed no reaction to the drug.

Blood was drawn at varying intervals following injection, as indicated in Table 9. In the single dose studies, specimens were weighed immediately, dried in an oven at 85°C. overnight, re-weighed, and ground. Suitable aliquots were taken for counting. In the case of patient No. 8, oxalated blood was measured for radioactivity directly, determinations being made on samples of one cubic centimeter.

Urine was collected at frequent intervals during the first day, and thereafter in pools covering 12 or 24-hour periods. The volume was measured and an

TABLE 2

*Patient No. 1. Total antimony excretion over a 7-day period. Dose, 18.77 mg. Sb.—
0.253 mg. per kg.*

HOURS t	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
0.5	0.80					
2	0.05	0.85				
4	0.17	1.02				
8	0.17	1.19				
16	0.62	1.81				
24	0.14	1.95	0.31		2.26	12.0
36	0.39	2.34				
48	0.32	2.66	0.09	0.40	3.06	16.3
60	0.21	2.87				
72	0.39	3.26	0.04	0.44	3.70	19.7
84	0.20	3.46				
96	0.27	3.73	0.13	0.57	4.30	22.9
108	0.33	4.06				
120	0.18	4.24	0.05	0.62	4.86	25.9
132	0.21	4.45				
144	0.15	4.60	0.05	0.67	5.27	28.0
156	0.14	4.74				
168	0.14	4.88		0.67	5.55	29.6

TABLE 3

*Patient No. 2. Total antimony excretion over a 7-day period. Dose, 37.5 mg. Sb.—
—0.570 mg. per kg.*

HOURS t	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
0.5	0.95					
2	0.97	1.92				
4	0.57	2.49				
8	0.15	2.64				
10	0.28	2.92				
24	1.37	4.29	0.69		4.98	13.3
36	0.45	4.74				
48	0.51	5.15	0.59	1.28	6.53	17.4
60	0.57	5.82				
72	0.23	6.05	0.32	1.60	7.65	20.4
84	0.36	6.41				
96	0.43	6.84	0.30	1.90	8.74	23.3
108	0.25	7.09				
120	0.32	7.41	0.27	2.17	9.58	25.6
132	0.42	7.83				
144	0.17	8.00	0.09	2.26	10.26	27.4
156	0.21	8.21				
168	0.14	8.35	0.13	2.39	10.74	28.6

aliquot taken for analysis. Analysis was carried out on one cubic centimeter of urine which was allowed to dry before the determination.

Stools were collected in 24-hour lots. Specimens were diluted, thoroughly mixed in a Kahn shaker, and a 25 ml. aliquot was dried, weighed, and submitted for counting.

TABLE 4
Patient No. 3. Total antimony excretion over a 27-day period. Dose, 56.5 mg. Sb.—
0.64 mg. per kg.

TIME	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
1 hr.	1.81					
1-24 hrs.	2.69	4.50	0.44		4.94	13.5
1-2 days	1.64	6.14	0.09	0.53	6.67	18.3
2-3 days	1.55	7.69	0.27	0.80	8.49	23.3
3-4 days	1.01	8.70	0.22	1.02	9.72	26.6
4-5 days	0.72	9.42	0.19	1.21	10.63	29.2
5-6 days	0.69	10.11	0.05	1.26	11.37	31.2
6-7 days	1.08	11.19	0.08	1.34	12.53	34.4
7-8 days	1.00	12.19	0.11	1.45	13.64	37.4
8-9 days	0.79	12.98	0.11	1.56	14.54	40.0
9-10 days	0.92	13.90	0.09	1.65	15.55	42.7
10-11 days	0.66	14.56	0.11	1.76	16.32	44.8
11-12 days	0.78	15.34	0.04	1.80	17.14	47.0
12-13 days	1.62	16.96	0.04	1.84	18.80	51.6
13-14 days	1.79	18.75	0.04	1.88	20.63	56.6
14-15 days	0.61	19.36	0.11	1.99	21.35	58.5
15-16 days	0.00	19.36	0.04	2.03	21.39	58.6
16-17 days	0.62	19.98	0.04	2.07	22.05	60.5
17-18 days	0.43	20.41	0.07	2.14	22.55	61.8
18-19 days	0.60	21.01	0.02	2.16	23.17	63.5
19-20 days	0.40	21.41	0.01	2.17	23.58	64.7
20-21 days	0.45	21.86	0.05	2.22	24.08	66.2
21-22 days	0.54	22.40	0.06	2.28	24.68	67.7
22-23 days	0.39	22.79	0.06	2.34	25.13	68.9
23-24 days	0.60	21.01	0.07	2.41	25.56	70.0
24-25 days	0.40	21.41	0.03	2.44	25.94	71.2
25-26 days	0.33	21.83	0.01	2.45	26.28	72.1
26-27 days	0.31	24.14	0.09	2.54	26.68	73.3

Determinations on blood samples can be reproduced by this technique to within ± 0.01 micrograms; those on urine and stool samples to within ± 5 micrograms.

RESULTS

The data on the urine and stool excretion of the 7 subjects receiving a single dose are presented in Tables 2 through 8. The total excretion is plotted, as percent per 24-hour period, in Graph I.

From the data given in the tables, the percentage of antimony eliminated by the urinary and intestinal routes may be calculated. Using 100 percent as the

TABLE 5

Patient No. 4. Total antimony excretion over a 7-day period. Dose, 49.55 mg. Sb.—
0.642 mg. per kg.

HOURS †	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
1	1.51					
2	0.39	1.90				
3	0.30	2.20				
4	0.22	2.42				
5	0.35	2.77				
6	0.16	2.93				
7	0.13	3.06				
8	0.17	2.23				
12	0.49	3.72				
16	0.43	4.15				
24	0.15	4.30	0.68		4.98	10.1
36	1.36	5.66				
48	0.62	6.28	0.68	1.36	7.64	15.4
60	0.18	6.46				
72	0.40	6.86	0.09	1.45	8.31	16.8
96	0.28	7.14	0.09	1.54	8.68	17.5
108	0.37	7.51				
120	0.73	8.24	0.09	1.63	9.87	19.9
132	0.53	8.77				
144	0.20	8.97	0.09	1.72	10.69	21.6
168	0.19	9.16	0.14	1.86	11.02	22.4

total amount eliminated during the period of observation, this partition is as follows:

PATIENT NO.	PERIOD OF OBSERVATION IN DAYS	PERCENT OF TOTAL ELIMINATED BY:	
		Urine	Feces
1	7	88	12
2	7	78	22
4	7	84	16
5	6	78	22
6	5	78	22
7	7	82	18
8	45	77	23

Two patients (patients Nos. 6 and 7) vomited shortly after the injection. In each of these the vomitus was saved and analyzed by the method used for stools. Patient No. 6 had eliminated 1.2 percent, and patient No. 7, 0.1 percent of the total dose received, within a half hour after administration. In one patient the vomitus was bile tinged, in the other it was colorless. That the antimony was probably eliminated through the stomach wall, and not by way of the biliary tract, is further suggested by the following observation. A rhesus tube was passed to the duodenum in another volunteer and visualized by X-ray.

TABLE 6

Patient No. 5. Total antimony excretion over a 6-day period. Dose, 50.0 mg. Sb.—
0.781 mg. per kg.

HOURS t	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
1	1.59					
2	0.26	1.85				
3	0.32	2.17				
4	0.25	2.42				
5	0.29	2.71				
6	0.16	2.87				
7	0.14	3.01				
8	0.37	3.38				
16	0.45	3.83				
24	0.42	4.25	1.14		5.39	10.8
36	1.50	5.75				
48	0.73	6.48	0.83	1.97	8.45	16.9
60	0.95	7.43				
72	0.77	8.20	0.42	2.39	10.59	21.2
84	0.79	8.99				
96	0.57	9.56	0.37	2.76	12.32	24.6
108	0.67	10.23				
120	0.61	10.84	0.38	3.14	13.98	28.0
132	1.0	11.84				
144	0.37	12.21	0.27	3.41	15.62	31.2
168			0.16	3.57	15.78	31.6

TABLE 7

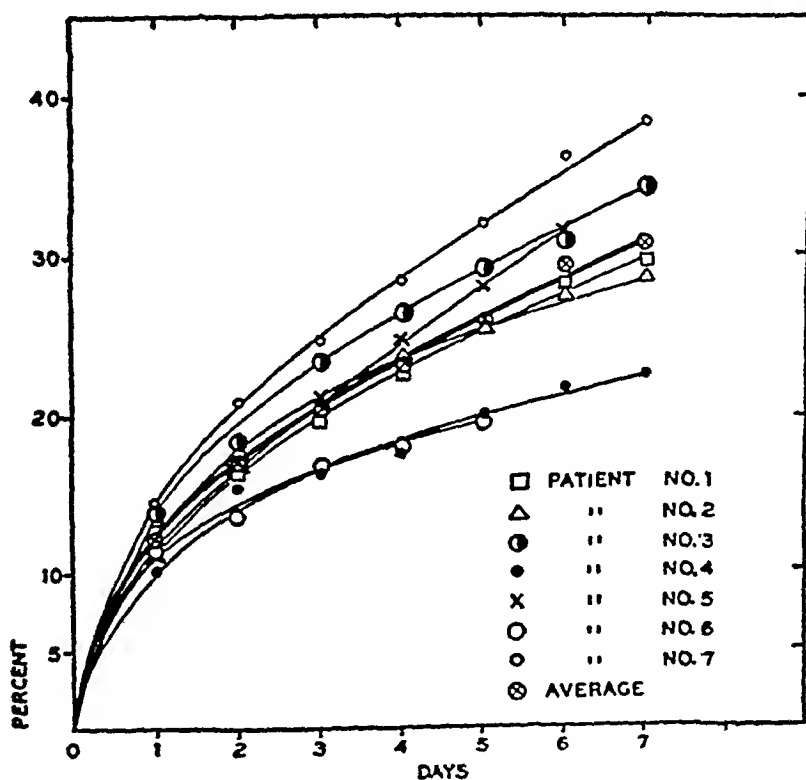
Patient No. 6. Total antimony excretion over a 5-day period. Dose, 122.8 mg. Sb.—
1.502 mg. per kg.

HOURS t	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
1	3.64					
6.25	2.75	6.39				
7.5	0.77	7.16				
8	0.33	7.49				
8.5	0.20	7.69				
13.5	0.99	8.68				
16	0.99	9.67				
20	0.51	10.18				
24	0.66	10.84	1.64		12.48	11.4
33	0.49	11.33				
38	0.25	11.58				
48	0.19	11.77	1.64	3.28	15.05	13.5
55	0.64	12.41				
72	1.99	14.40	1.65	4.93	19.33	16.9
84	0.53	14.93				
96	0.76	15.69	0.05	4.98	20.67	18.0
108	1.01	16.70				
120	1.15	17.85	0.12	5.10	22.95	19.9

TABLE 8

Patient No. 7. Total antimony excretion over a 7-day period. Dose, 120 mg. Sb.—
1.60 mg. per kg.

HOURS t	URINE		STOOL		TOTAL	
	mg. Sb.	Total	mg. Sb.	Total	mg. Sb.	Percent
1	3.72					
2	1.13	4.85				
3	1.40	6.25				
4	0.39	6.64				
6	1.82	8.46				
8	1.00	9.46				
12	3.32	12.78				
16	1.23	14.01				
24	1.93	15.94	1.20		17.14	14.4
36	2.19	18.13				
48	2.50	20.63	2.96	4.16	24.79	20.8
60	2.04	22.67				
72	2.15	24.82	0.55	4.71	29.53	24.7
84	1.78	26.60				
96	2.06	28.66	0.55	5.26	33.92	28.4
108	2.10	30.76				
120	1.80	32.56	0.50	5.76	38.32	32.0
132	1.56	34.12				
144	2.04	36.16	1.63	7.39	43.55	36.4
168	1.41	37.57	0.94	8.33	45.90	38.4



GRAPH I: THE TOTAL ANTIMONY EXCRETION OF 7 PATIENTS WHO RECEIVED A SINGLE DOSE OF TARTAR EMETIC INTRAVENOUSLY

One hundred milligrams of tartar emetic (36.5 mg. Sb.) was then administered by vein. All the bile was collected for an hour, after which magnesium sulfate was administered through the tube and the bile again withdrawn. The total recovery of antimony was only 0.05 percent of that injected.

Table 9 shows the blood levels of antimony for 7 patients at varying intervals following the intravenous injections; those of 6 patients receiving single doses are presented in graphic form, for the first 12 hours, in Graph II.

TABLE 9

Blood antimony, in micrograms per gram wet blood, at various times following intravenous administration

(Micrograms per gm. wet blood $\times 1000/1.06$ = micrograms per litre)

TIME	PATIENT NO.						
	1	2	4	5	6	7	8
0*	(2.9)*	(6.5)*	(7.3)*	(8.9)*	(17.1)*	(18.2)*	(3.24)*
5 min.					1.47		
10 min.					1.17		
20 min.					1.01		
30 min.	0.29	1.34	0.65	0.42	0.80	0.88	
1 hour	0.21	0.45	0.32	0.35	0.51	0.81	
2 hours	0.15	0.32	0.36	0.19	0.45	0.53	0.14
4 hours	0.08	0.20	0.29	0.15		0.27	0.14
8 hours			0.20	0.11	0.14	0.22	
12 hours			0.14	0.08	0.10	0.11	
16 hours	0.07		0.18	0.05	0.09	0.13	
24 hours	0.04	0.07	0.01	0.06	0.07	0.00	0.06
48 hours	0.01	0.04	0.00	0.03	0.04	0.05	0.03†
50 hours							0.36
72 hours	0.06	0.03	0.04	0.00	0.04	0.05	0.16
96 hours		0.01	0.03	0.01	0.04	0.03	0.11‡
98 hours							0.45
120 hours	0.00		0.03	0.03	0.10	0.04	0.25
144 hours	0.04		0.07	0.02	0.07	0.08	0.21‡
146 hours							0.53
168 hours						0.02	

* "Theoretical initial level" calculated on the assumption that blood comprises 8.8 per cent of body weight.

† 36.5 mg. given immediately after this determination.

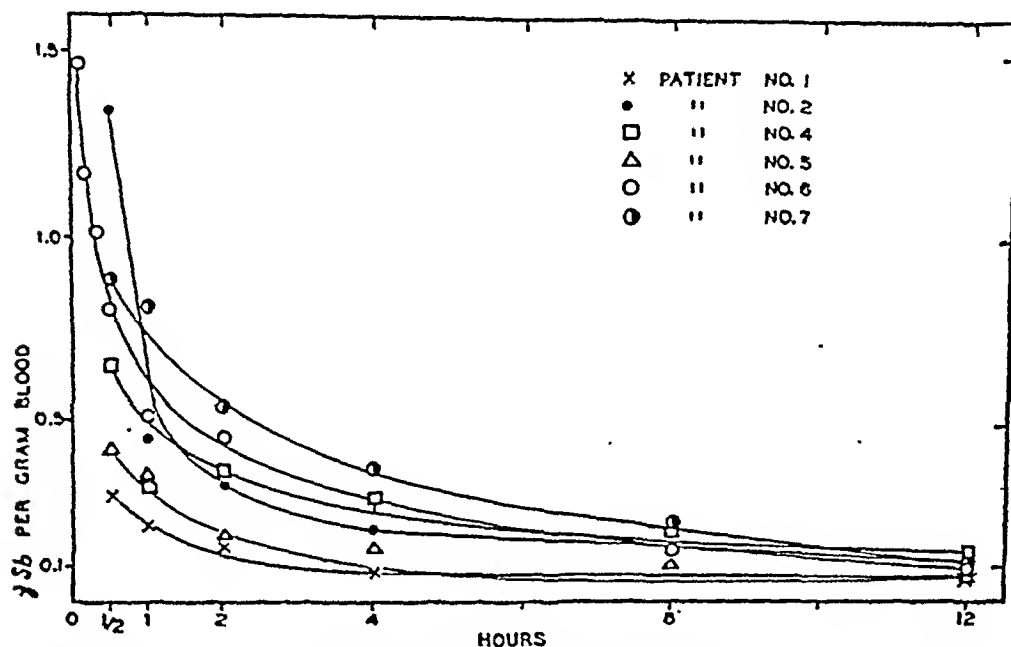
‡ 54.7 mg. given immediately after this determination.

In Table 10 are presented the data on antimony excretion for patient No. 8. It will be noted that the second and succeeding doses were given immediately after the collection period listed in the same horizontal column. "Percent" is arbitrarily taken to refer to percent of total antimony present in the body at the time of the last injection.

REVIEW OF LITERATURE

With regard to the distribution of antimony in the excreta following intravenous administration, there is little information in the literature. Faust and

Meleney (4) stated that "It is known that a large proportion of the antimony is excreted by way of the intestine." Similarly Chopra (5) stated that "Antimony salts are mainly excreted by the stomach, the intestines, and the kidneys." Neither paper gives quantitative data nor the source of the information.



GRAPH II: THE BLOOD ANTIMONY LEVELS OF 6 PATIENTS WHO RECEIVED A SINGLE DOSE OF TARTAR EMETIC INTRAVENOUSLY

Khalil (6) studied the urinary and fecal excretion of antimony following fuadin administration in full therapeutic courses. He reported the antimony output in 4 patients for varying periods of time, with findings as follows:

PATIENT NO.	DAYS AFTER LAST INJECTION	TOTAL AMOUNT ELIMINATED (Percent of dose)	PERCENT OF TOTAL ELIMINATED BY:	
			Urine	Feces
1	5	48.2	93	7
2	5	60.1	92	8
3	5	30.4*	93*	7*
	28	53.6	96	4
4	5	30.7*	93*	7*
	37	53.4	96	4

* Percentage calculated from tables in text.

Studies on the first 2 patients were stopped on day 5, those on the latter 2 were carried through days 28 and 37, respectively. The ratio of fecal to urinary excretion thus decreases as smaller amounts are recovered. The very low absolute values obtained by this author in the longer experiments may be

TABLE 10
Patient No. 8. Total antimony excretion over a 45-day period. Dose, 656.45 mg. Sb.—
10.1 mg. per kg. in therapeutic course

DAYS	URINE		STOOL		TOTAL		DOSE*
	mg. Sb.	Total mg.	mg. Sb.	Total mg.	mg. Sb.	Percent	mg. Sb.
0-1½	1.60						18.25
1½-2	0.88						
2-3	1.75		0.13				
3-4	2.93	7.16	0.28	0.41	7.57		36.5
4-6	12.65	19.81	1.17	1.58	21.39		54.7
6-8	22.06	41.87	1.53	3.11	44.98		54.7
8-10	23.23	65.10	4.28	7.39	72.49		54.7
10-12	24.84	89.94	5.57	12.95	102.89		54.7
12-14	34.19	124.13	4.96	17.91	142.04		54.7
14-16	45.52	169.65	6.40	24.31	193.96		54.7
16-18	32.40	202.05	8.35	32.66	234.71		54.7
18-20	30.50	232.55	6.76	39.42	271.97		54.7
20-22	19.61	252.16	6.15	45.57	297.73		54.7
22-24	37.73	289.89	9.81	55.38	345.27		54.7
	24.99	314.88	7.31	62.69	377.57		54.7
Total dose.....							656.45
24-25	17.22		3.74		20.96	7.5	
25-26	5.96	23.18	5.00	8.74	31.92	11.4	
26-28	18.83	42.01	1.53	10.27	52.28	18.7	
28-30	15.33	57.34	4.28	14.55	71.89	25.8	
30-32	12.25	69.59	5.57	20.12	89.71	32.1	
32-34	14.20	83.79	4.96	25.08	108.87	39.1	
34-36	16.20	99.99	6.40	31.48	131.47	47.2	
36-38	9.18	109.17	8.35	39.83	149.00	53.5	
38-40	13.80	122.97	6.76	46.59	160.56	60.8	
40-42	5.38	128.35	6.15	52.74	181.09	65.0	
42-44	5.79	134.14	9.81	62.55	196.69	70.5	
44-45	2.71	136.85	5.43	67.98	204.83	73.5	

* "Dose" was given immediately after the collections whose values are listed in the corresponding column. Thus at end of day 2, 7.57 mg. Sb. had been excreted; 36.5 mg. were given, and subsequent excreta added to day 3, etc.

attributable in part to the limitations of the method of analysis (Beam and Freak). Comparable results on our patient No. 8 are as follows:

DAYS AFTER LAST INJECTION	TOTAL AMOUNT ELIMINATED (Percent of dose)	PERCENT OF TOTAL ELIMINATED BY:	
		Urine	Feces
5	22.2	82	18
21	73.5	77	23

With regard to the rate of elimination of antimony by the kidneys, all reports agree that trivalent antimony is excreted more slowly than the pentavalent form.

There is considerable difference of opinion, however, as to the rate of excretion of the various trivalent compounds.

Hassan (7, 8) reported the excretion of fuadin and tartar emetic following single doses administered intravenously. His cases are separated into those with normal and abnormal kidney function, as judged by the urea clearance test. His findings for the 24-hour excretion were as follows:

Fuadin	Normal kidneys:	8.6-10.8 percent
	Abnormal kidneys:	6.5- 7.3 percent
Tartar emetic	Normal kidneys:	11.3 and 13.8 percent
	Abnormal kidneys:	11.0 and 12.7 percent

The higher figures which he originally reported for fuadin excretion were retracted in the later paper in the belief that the drug had contained some pentavalent antimony.

Boyd and Roy (9) studied 2 patients for 3 days following intravenous administration of sodium antimonyl tartrate. The total urinary excretion found in this period was 4.5 and 3.5 percent, respectively.

Goodwin and Page (10) gave fuadin in single doses to human subjects and reported excretion of 18 to 35 percent in 24 hours.

Alves *et al.* (11) treated cases of schistosomiasis with rapid courses of sodium antimonyl tartrate. They studied the urinary antimony excretion in 8 patients over a period of 72 hours, including the 2 days of treatment. The excretion in these subjects varied from 18 to 28 percent of the total injected antimony.

In the dog, Weese (12) reported 3-day excretion of antimony following the use of fuadin administered intramuscularly. The amounts varied from 20 to 52 percent. With higher doses (2.13 mg. per kilogram) the percent excreted the first day was considerably higher than it was with the lower doses (0.85 mg. per kilogram). Brady *et al.* (13) treated 4 dogs with tartar emetic at a dosage level of 0.8 mg. of antimony per kilogram of body weight. In the following 36 hours, these animals excreted in the urine from 4 to 21.2 percent of the antimony administered. The above data are summarized in the following table:

AUTHOR	METHOD	SUBJECT	DRUG	DOSAGE	HOURS	PERCENT EXCRETED
Hassan	Gutzeit	Man	Fuadin	"Usual therapeutic"	24	6.5-10.8
		Man	Tartar emetic	"Usual therapeutic"	24	11-32
Boyd and Roy	Gutzeit	Man	Sodium anti-monyl tartrate	18.77 mg. Sb.	72	4.5
		Man	Sodium anti-monyl tartrate	22.5 mg. Sb.	72	3.5
Goodwin and Page	Polarographic	Man	Fuadin	42.5 mg. Sb.	24	25.7
		Man	Fuadin	8.5 mg. Sb.	24	18-35
Alves <i>et al.</i>	Not stated	Man	Sodium anti-monyl tartrate	254-348 mg. Sb.	72	18-28
		Dog	Fuadin	0.85 mg. Sb./kg.	72	20-41
Weese	Not stated	Dog	Fuadin	2.13 mg. Sb./kg.	72	25-52
		Dog	Tartar emetic	0.80 mg. Sb./kg.	36	4-21.2
Brady <i>et al.</i>	Radio-antimony	Dog	Tartar emetic			

Our own results (Tables 2-8 and Graph I) may be summarized as follows:

DOSAGE	HOURS	PERCENT EXCRETION	PERCENT EXCRETION
		<i>Average</i>	<i>Range</i>
18.77 to 120 mg. Sb. or 0.253 to 1.6 mg. Sb./kg.	24	12.2	10.1-14.4
	48	16.9	13.5-20.8
	72	20.4	16.8-24.7
	168	30.7	22.4-38.4
	(7 days) 27 days	73.3	

There was no apparent relation between the size of dose and the rate of excretion nor was there any correlation observed between previous antimony dosage and rate of elimination of the radioactive dose. One patient (No. 7) continued to receive his therapeutic course of non-radioactive antimony beginning the second day after the test dose. No effect on the elimination of the labelled drug was observed. This fact proved to be of importance in the interpretation of the relation between blood level and rate of excretion; the point is further discussed in the paper by Forbush *et al.* (14).

As has been stated, the percent antimony excretion of patient No. 8 was calculated on the basis of the total amount present in the body immediately following the last dose. This patient had excreted 7.5 per cent in 24 hours, 29 percent in 7 days, and a total of 73.5 percent in 21 days.

Brady *et al.* (13) reported the only available studies of consecutive blood levels following single intravenous doses of antimony. A comparison of their findings with those in a human volunteer is given by Forbush *et al.* (14). It may be stated here that the mechanism of removal of antimony from the blood of man appears, with the information at hand, to be identical with that in the dog.

DISCUSSION

Very little is known of the "intermediate metabolism" of tartar emetic from the time that it is injected into the vein until it appears in the stools and urine. Because of its strongly acid reaction, it is inconceivable that it can remain in the blood as such more than momentarily. Clinical evidence supports this reasoning. In administering courses of treatment, we have repeatedly observed that the immediate reactions, cough and vomiting, can be minimized or eliminated by slowing down the rate of injection; and individual patients may show striking differences in the "maximum tolerated rate" of injection. In particular, it was noted above that patient No. 1 suffered from nausea, and patient No. 4 developed cough during injection. In both these men, the injection was continued to completion at a slower rate, and the symptoms disappeared even while more antimony was being given.

These observations, however, give no evidence as to whether the drug is changed to a less toxic form, which is then handled as a diffusible metabolite, or is immediately removed from the blood stream. The final answer to this

question must await correlation between *in vivo* and *in vitro* studies. The present data show, however, the extreme rapidity with which removal from blood takes place. In fact, the rate of fall of blood level alone is sufficient to account for the clinical observations. In one patient (patient No. 6) blood was drawn 5 minutes after the end of the injection. The antimony level (1.47 micrograms per gram wet blood) had dropped to 9 percent of the "theoretical initial level" calculated from dose and blood volume (see Table 9, "time 0"). Initial determinations on 5 other patients were made 30 minutes after injection. The values were 5, 5, 10, 11, and 20 percent respectively, of the "theoretical initial level." Thus the observed changes in blood antimony discussed in this study represent roughly the final 10 percent of the drug to be disposed of.

The relation of blood antimony levels to efficiency of therapy has not been established. Inasmuch as many of the organisms killed by antimonials, schistosomes, trypanosomes, and *Dirofilaria*, are blood stream inhabitants, it appears not unlikely that a direct relationship exists.

On the experimental side, Cowie *et al.* (15) have presented evidence suggesting that it is possible by daily repeated doses of a trivalent antimonial to raise progressively the "basic" blood level of antimony. The method does not allow determination of the type of compound or the valence of the antimony. Clinically, it has been found repeatedly that increase in the frequency and size of doses of antimonials brings about increase in the number of cured cases. This has been the experience of one of us (16) as well as that of Brown (17) and of Culbertson and Rose (18, 19). Alves *et al.* (11) have reported the most rapid course of therapy thus far applied in clinical practice; and their early results suggest a very high percentage of cures, despite a very low total antimony dosage.

The extremely rapid fall of blood antimony following intravenous administration, as reported in this communication, may explain the failures in therapy with infrequent dosage, inasmuch as virtually no blood level is maintained. By analogy with the dog, it would appear possible to raise the sustained blood antimony concentration in man to any convenient level, within the limitations of the toxicity of the drug. The early blood antimony values in patient No. 8 show a progressive step-wise increase. In the paper by Forbush *et al.* (14), the theoretical implications of this finding are discussed, and an attempt is made to derive a general rule for rational antimony therapy.

SUMMARY

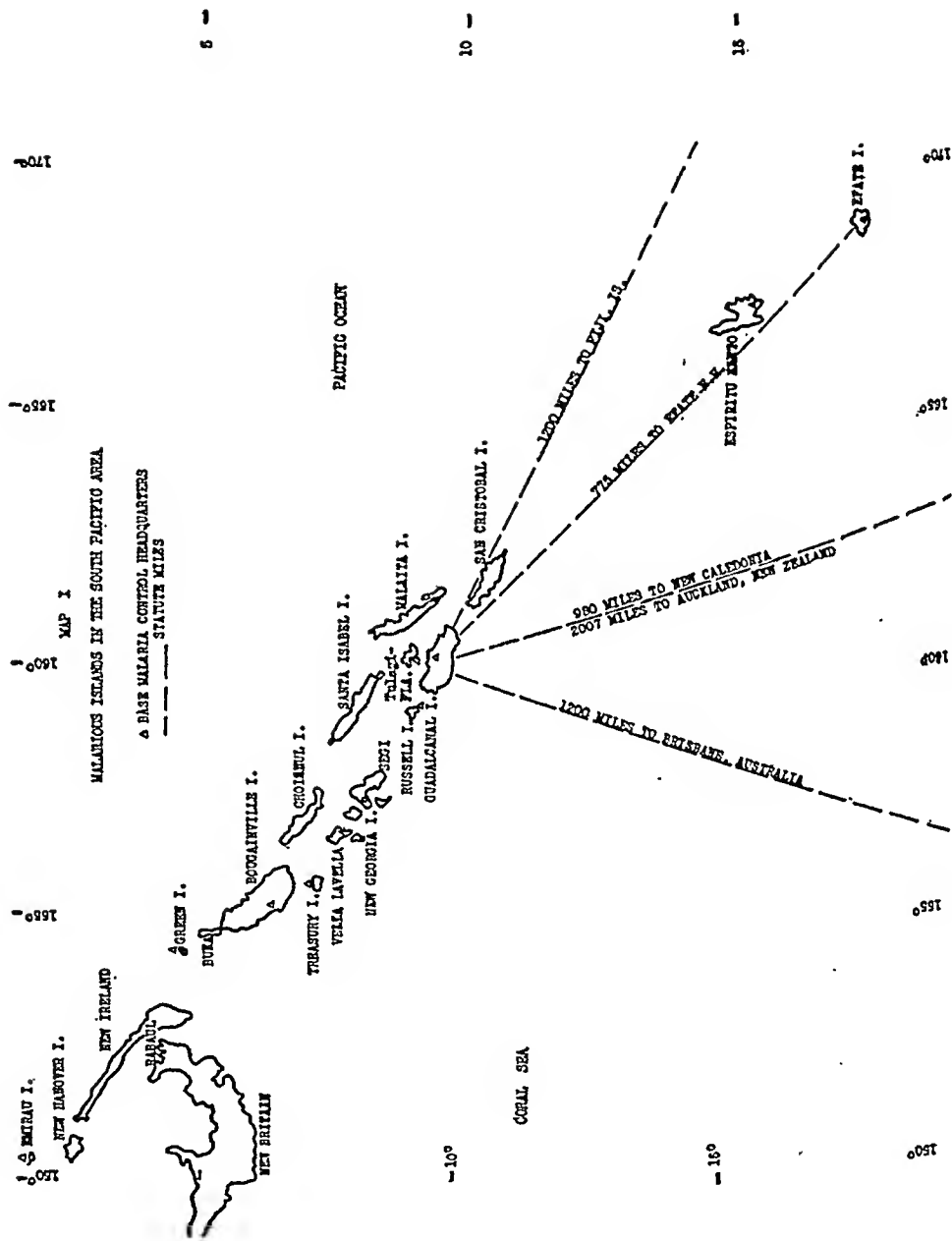
1. The use of radioactive antimony provides a useful tool in the study of the metabolism of the antimonials used in the treatment of tropical diseases.
2. Following a single intravenous dose of tartar emetic, there is rapid elimination by urine and feces for the first 2 days, followed by a slower and relatively steady rate of elimination for the following 5 days. Approximately 80 percent is eliminated by way of the urine, and 20 percent by the gastro-intestinal tract.
3. Approximately 12 percent of the antimony is eliminated in 24 hours; 30 percent in a week; and, in one patient, 73 percent of the antimony was eliminated in 4 weeks.

4. The blood antimony level falls precipitously following injection. There is evidence that the immediate toxic symptoms of antimony therapy are due to injection at a rate too rapid to allow for this drop. The poor results of therapy by infrequent doses may be attributable to the extremely low blood level which persists after a brief interval.

5. Analogous results of multiple injections in man and the dog suggest that the basic blood level of antimony may be controlled by adjusting the size and frequency of doses.

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MALARIA AND OTHER INSECT-BORNE DISEASES IN THE SOUTH PACIFIC CAMPAIGN

1942-1945

A SERIES OF FOUR PAPERS

TABLE OF CONTENTS

	<i>Page</i>
FOREWORD.....	iii
Brigadier General James S. Simmons	
ACKNOWLEDGMENT.....	v
I. GENERAL ASPECTS AND CONTROL MEASURES	
P. A. Harper, E. T. Lisansky and B. E. Sasse,	
A. Introduction.....	1
B. Early Epidemics.....	1
C. Physical Geography, History, Period of Occupation.....	5
D. Area Organization and Procedure.....	9
1. Legal Basis and Development of Area Organization.....	10
2. Extension of Work to Control of Filariasis, Dengue, Tsutsugamushi Disease and Rodent Control.....	13
3. Procurement and Assignment of Personnel.....	15
4. Supplies and Equipment.....	20
E. Base or Island Organization and Procedure.....	21
1. The Work of the Malariologist.....	21
2. The Malaria Survey Detachment.....	26
3. The Malaria Control Detachment.....	27
4. Troop Unit Anti-Malaria Organization.....	29
5. Division Malaria and Insect Control Organization.....	30
F. The Training and Education Program.....	31
1. The Work of the Area Training and Education Staff.....	31
2. Training Program on Each Island or Base.....	33
G. Control Measures.....	37
1. Individual Protective Measures.....	37
2. Segregation of Natives—Selection of Camp Sites.....	38
3. Larvicidal Work—Ground Application.....	42
4. Larvicidal Work—Airplane Application.....	46
5. Measures Directed Against Adult Mosquitoes.....	49
6. Semipermanent and Permanent Control Work.....	51
7. Suppressive Medication—Atabrine.....	56
H. Comment.....	60
Appendix I. Standard Operating Procedure for Control of Malaria and Other Insect-Borne Diseases during a Combat Operation.....	64
II. EPIDEMIOLOGY OF INSECT-BORNE DISEASES IN ARMY TROOPS	
W. G. Downs, P. A. Harper, and E. T. Lisansky	
A. Explanation of Graphs and History of Individual Bases.....	69
B. Malaria Experience of Certain Army Units.....	81
C. Dengue Fever.....	86
D. Other Insect-borne Diseases.....	87
E. Comment.....	87
F. Summary.....	89

¹All of the Contributors to this series of papers were commissioned officers in the medical Corps or Sanitary Corps, Army of the United States.

III. ENTOMOLOGY

P. W. Oman and L. D. Christianson

A. Topography and Climate.....	92
B. Entomological Problems Encountered.....	96
1. Malaria Vectors.....	96
2. Dengue Vectors.....	100
3. Filaria Vectors.....	100
4. Pest Mosquitoes.....	101
5. Flies.....	102
6. Other Arthropods of Importance.....	103
C. Organization of Survey Activities.....	103
D. Survey Duties and Procedure.....	105
1. Initial Surveys.....	105
2. Maps and Mapping.....	108
3. Integration of Survey and Control Activities.....	110
4. Routine Evaluation of Control Activities.....	111
5. Records and Reports.....	112
6. Policy Determination.....	113
E. Investigational Projects.....	114
F. Factors Limiting Effectiveness of Program.....	115
IV. PARASITOLOGICAL OBSERVATIONS ON MALARIA IN NATIVES AND TROOPS AND ON FILARIASIS IN NATIVES	
N. D. Levine and P. A. Harper	
A. Surveys of Natives.....	119
1. Malaria in Natives.....	120
2. Filariasis in Natives.....	124
B. Plasmodium Species in Troops.....	125
C. Discussion.....	127
D. Summary.....	127

FOREWORD

Malaria was the most serious health hazard experienced by American troops in the South Pacific area during World War II. In spite of the fact that for many years we have known much about the etiology, treatment and prevention of malaria and the Army and Navy have long been aware of its military importance, this disease attacked approximately 100,000 men of the armed forces in the South Pacific, and for a short time jeopardized the success of the military campaign in that area. This situation, including difficulties in the transport of malaria control supplies and inadequate local provision for malaria control organization and malaria discipline among troops, was more serious in the Pacific during the early period of the war than in any other theatre or at any other time.

Fortunately, this undesirable situation was soon corrected and commanders, previously unimpressed with the military importance of malaria, took active steps to wage a campaign against the disease. Priorities were established which enabled the War Department to supply the specialized personnel required to deal with the problem, consisting of malariologists, malaria survey detachments, malaria control detachments, and other medical and sanitary personnel. Priorities were also set up which allowed the movement of malaria control supplies from the docks in San Francisco, and the Army and Navy cooperated in the development of an area-wide all services organization for the control of malaria which also proved effective in the control of other arthropod-borne diseases, including dengue, filariasis and scrub typhus.

This series of papers, I through IV, deals primarily with the malaria problem and includes brief discussions of the other insect-borne diseases. It summarizes the work and reports of many individuals and it attempts to present an area-wide perspective of the malaria control program which was spread over many thousands of miles on the eleven malarious and numerous non-malarious bases. The first paper deals with the background of the epidemics of insect-borne diseases and with the organization, the training program, and the control measures which were employed. Papers II, III, and IV describe respectively the epidemiological factors, the entomological problems, and the parasitological data of this important wartime malaria control program. These papers have been edited by Lt. Colonel Paul Harper, who was Chief Army Malariologist in the South Pacific Area and in general charge of the malariologists and malaria survey and control units in that area. The Medical Department of the Army is proud of the large group of its personnel who worked so effectively to bring malaria under control in such places as Guadalcanal, where this disease at one time severely threatened our Pacific operations. These men with their collaborators in the Navy did much to hasten the surrender of the Japanese.

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ACKNOWLEDGEMENT

We are grateful to Brigadier General Earl Maxwell, United States Army, Surgeon, United States Army Forces in South Pacific Area; to Captain Arthur H. Dearing, United States Navy, Force Medical Officer, South Pacific Area; to his successor, Captain Frederick R. Hook, United States Navy, and to Brigadier J. W. Twigg, Director Medical Services, New Zealand Expeditionary Forces, Pacific, for their constant support of the South Pacific Malaria Control Organization and its work as described in the following pages.

We wish it were possible to give recognition to all those who aided in this work. Unfortunately this cannot be done. Special acknowledgement is made of the noteworthy contributions of Commander James J. Sapero, Medical Corps, United States Navy, Malaria and Epidemic Control Officer, South Pacific Area and of his successor, Commander F. A. Butler, United States Navy.

Appreciation is expressed to Colonel E. G. Sayers, Medical Corps, New Zealand Expeditionary Forces in Pacific for assistance developing from his special knowledge of tropical diseases in this area.

Acknowledgement is made to authorities in the Preventive Medicine Service of the Office of the Surgeon General, United States Army, including Brigadier General James S. Simmons, United States Army, Chief of Preventive Medicine Service, Office of the Surgeon General; Colonel Paul F. Russell, M.C., A.U.S., former Director Tropical Disease Control Division, Office of the Surgeon General; to his successor Lt. Colonel O. R. McCoy and to Colonel W. A. Hardenbergh, Director of Sanitary Engineering Division, Office of the Surgeon General. These officers contributed to the development of the basic organization of the malaria control program and provided continued support and assistance.

MALARIA AND OTHER INSECT-BORNE DISEASES IN THE SOUTH PACIFIC CAMPAIGN

1942-1945

I. GENERAL ASPECTS AND CONTROL MEASURES

P. A. HARPER,* E. T. LISANSKY² AND B. E. SASSE³

A. INTRODUCTION

The epidemics of malaria and other tropical diseases which afflicted our troops in the South Pacific as they moved against the advancing Japanese called forth an organization and methods of prevention which proved that such diseases need not jeopardize the success of military operations in the tropics. The South Pacific Malaria and Insect Control Organization¹ was a joint Army-Navy-Allied group, and while these papers are concerned primarily with the organization of Army personnel, it is to be emphasized that the cooperative spirit within this joint service organization contributed immeasurably to its success. The broad outline of the problem has been described by Sapero and Butler, (1)² and the control program, by Butler (2). This and subsequent sections in this series of papers make free use of material from a confidential report, since unclassified.³

Both published and unpublished reports of several individuals are cited in this paper but this has not been possible in a larger number of instances. It is hoped that those who were associated with particular phases of the program will record their own observations.

B. EARLY EPIDEMICS

The causative factors for repeated and crippling epidemics of malaria among troops were present in the South Pacific Area. The indigenous natives, the European whites and the invading Japanese were heavily infected with malaria and constituted an ever present seed bed. There was an exceedingly efficient vector, *Anopheles (Myzomia) farauti* Laveran⁴ and other members of this complex. Our troops were completely susceptible not only to malaria but to dengue, filariasis and tsutsugamushi fever, each of which caused localized but serious problems.

Malaria reached epidemic proportions among our forces on Efate and Guadalcanal. Lesser outbreaks occurred on Espiritu Santo, Tulagi-Florida, the Russell Islands, and Munda, New Georgia. No serious outbreaks of malaria occurred on Treasury, Bougainville, Green or Emir Islands which were the last four bases

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¹ This organization was first known as "South Pacific Malaria Control," and later as "South Pacific Malaria and Epidemic Control."

² Numerals in parenthesis indicate literature cited.

⁴ Harper, P. A., Butler, F. A., Lisansky, E. T., and Speck, C. D., Malaria and Epidemic Control in the South Pacific Area, 1942-1944. Reproduced by Engineers, Headquarters, South Pacific Base Command, 2379 January 1945.

⁴ Also erroneously referred to as *A. punctulatus* Doenitz and *A. punctulatus* var. *moluccensis* (Sw. and Sw.).

to be occupied because the malaria control program on these bases was adequately organized and supported from the time of the initial landings.

The epidemics at Efate and Gundleanal indicate the seriousness of the early situation on the first malarious bases to be occupied in this area (Table I).

Efate was occupied in March 1942 by a few hundred allied troops who landed to build an airfield and to forestall the southward advance of the Japanese. Their bivouac site near the airfield was surrounded by anopheline breeding swamps and streams. Native laborers employed on the project were encamped nearby. A Marine defense battalion enroute to a non-malarious base was diverted to Efate and landed in April 1942. Their supply of anti-malaria drugs was inadequate, and their bed nets were deep in the ship's hold and not available for two weeks. Night work was a military necessity. The malaria rate of 2600 per 1000 per

TABLE I
Early malaria rates per thousand per annum for all forces, Efate and Guadalcanal*

YEAR	MONTH	EFATE	GUADALCANAL
1942	April	2678	
	May	982	
	June	915	
	July	518	
	August		14
	September		177
	October		1664
	November		1781
	December		972
1943	January		1169
	February		878
	March		1052

* See Graphs I & III, Paper II.

annum on Efate in April 1942 ensued from the close association of unseeded troops, infected natives and anophelines.

The first troops on Guadalcanal in August 1942 found few mosquitoes and in the early weeks had little malaria. Native laborers and the Japanese were the reservoir of infection. *Anopheles farauti* found ideal breeding places in the myriad ruts and holes made by the occupying forces and rapidly produced enormous populations. Malaria control measures received scant attention in September and October of 1942 because of the desperate military situation. Combat conditions and a small perimeter contributed to the factors required for an epidemic; namely, susceptible troops, anopheline mosquitoes and a seed bed of malaria in natives and Japanese. A few cases of malaria appeared in September, the disease became epidemic in October, with a peak case rate of 1800 per 1000 per annum in November. The epidemic lasted for nearly a year and the case rate averaged more than 1000 per 1000 per annum for eight months. A large part of

the malaria seen throughout the duration of the South Pacific Campaign represented infections and relapses from infections contracted during these early days on Guadalcanal.

Malaria caused more than five times as many casualties in the South Pacific as did combat. It is estimated that a total of 100,000 individuals, Army, Navy, Marine and Allied, contracted malaria in this area. Each of these individuals had an average of nearly two attacks, thus doubling the loss of man days.

Heavily infected troop units which were sent from Guadalcanal to rear bases and ordered to stop suppressive atabrine averaged $1\frac{1}{2}$ to 2 attacks of malaria per man before resuming suppressive therapy.⁶ Entire divisions were rendered both less effective in combat and during the period of rehabilitation because of the number of men ill with malaria.

The urgent military situation and the shortage of supplies was partially responsible for these early malaria epidemics. It was necessary to occupy Efate, Santo and Guadalcanal months before the arrival of trained malaria control personnel and equipment, over a year before the new repellents and freon aerosol insecticide dispensers were available, at a time when the supply of quinine was limited and when atabrine was still an unfamiliar drug. Although it was known that malaria alone could incapacitate an army and although information existed on how to deal with the problem, this knowledge, for reasons here given, was not applied in the South Pacific Area until devastating epidemics of malaria made prompt action imperative. The concept of "malaria discipline" had not been developed and the prevailing attitude was well expressed by one high ranking officer on Guadalcanal who said, "We are out here to fight Japs and to hell with mosquitoes."

The Japanese who also suffered severely from malaria, were less successful in their control efforts. There is evidence that uncontrolled malaria, beriberi and dysentery were among the decisive factors which cost the Japanese the Guadalcanal and Munda campaigns. The few prisoners taken throughout the campaign were in general emaciated, suffering from dysentery or helminthic diseases and almost invariably malarious.

Examination of Japanese base areas in the Solomons revealed little evidence of semipermanent malaria control. A few ingenious and efficient knapsack sprayers and small quantities of larvacidal oils were found. The Japanese used bed-nets, a repellent cream whose active ingredient was oil of citronella, and a form of punk for producing smoke with mosquito repellent properties. Although the Japanese had a large quantity of both quinine and atabrine, it seemed likely from allied intelligence reports that the supply was often exhausted in isolated garrisons.

The weakening of Japanese resistance by disease was not a completely favorable circumstance. Highly malarious Japanese undoubtedly infected a large percentage of the *Anopheles* mosquitoes in their vicinity. During ground combat and after occupation of Japanese positions by Allied troops, infected mosqui-

⁶ See Paper II, Graphs VIII and IX of rates in Americal Division and in 147th Infantry during discontinuation of atabrine suppressive medication.

toes transmitted much malaria from Japanese to American troops. Japanese were in some instances the principal source of infection for sudden outbreaks of malaria in front-line American troops. A sharp outbreak of malaria on Bougainville affecting many hundreds of Allied troops several months after occupation was directly traceable to seizure of a front-line area on Torokina River recently held by Japanese.

Table II was compiled from captured Japanese medical reports⁶, for Japanese forces in the Solomons, New Britain, and New Guinea during the period December 1942 through February 1943. A translated Japanese Medical Service Report⁷ for this period is quoted: "At Rabaul . . . during the month (of February 1943) 32.4% (605 men) of the Hq Sig Unit became malaria patients and of the 41st Inf Regt, 22.09% (716 men)". During the month of April 1943 the total malaria rate for Rabaul and vicinity was 2053 per 1000 per annum, according to other captured documents.

Dengue fever reached epidemic proportions on Fiji, New Caledonia, Efate and Tulagi-Florida although its importance was obscured by the deluge of malaria. It caused illness in over 25 per cent of the military population on Santos in the

TABLE II
*Malaria in Japanese forces**

MONTH AND YEAR	STRENGTH	MALARIA RATE PER 1,000 PER ANNUM (NEW PATIENTS)	MALARIA DEATHS
December 1942.....	51,352	450	1
January 1943.....	61,501	1,095	8
February 1943.....	79,901	1,637	13

* Captured medical data, Solomons and Bismarck Archipelago, compiled by Commander F. A. Butler, M.C., U. S. N.

first half of 1943 and resulted in over 80,000 sick days on this base alone before it was brought under control (3).

An extremely severe epidemic of filariasis which occurred in the Samoan Defense Area led to the medical evacuation of many thousands of troops. This offered an unusual opportunity to study the beginning of this disease in freshly exposed adults. It was demonstrated that the disease syndrome called "Mumu" by the natives was an early manifestation of infection with *Wuchereria bancrofti*. This syndrome was characterized by localized swellings, retrograde lymphangitis, lymphadenitis and by genital manifestations (4, 5, 6, 7, 8). Volumes 42, 43 and 44 of the United States Navy Medical Bulletin contain nine papers on this epidemic of filariasis. The report by Byrd et al. (9) describes the entomological and parasitological investigations which were fundamental to controlling the disease. Subsequent surveys on nearly every South Pacific Base showed a high incidence of filarial infection in natives, Paper IV.

⁶ Harper et al. cited in footnote on page 1.

⁷ Allied Translator Intelligence Service, South West Pacific Area Current Translations No. 121 dated 28 May 1944.

Over 75 cases of tsutsugamushi disease occurred on Bougainville, 49 of which are reported in (10). Three isolated cases occurred on Santos⁸ and about 10 unconfirmed cases were reported from Munda, New Georgia.

The control of all these arthropod-borne diseases became the responsibility of the South Pacific Malaria and Insect Control Organization.

C. PHYSICAL GEOGRAPHY, HISTORY, PERIOD OF OCCUPATION

The genesis of the epidemics of insect-borne diseases that occurred in the South Pacific was found in the combined factors of physical geography, infected natives, a potent vector and the impact of military activities

The South Pacific Area eventually expanded to embrace 11 malarious bases in the New Hebrides, the Solomon Islands, Green Island and Emirú Island (St. Matthias group), the last 2 being part of the Bismarck Archipelago. The occupied malarious islands (map of malarious islands in the South Pacific Area, frontis-piece) form a long chain extending from southeast to northwest, with Emirú 1°8' S.L. and 150° E.L. at the northwest end and Efate 17°30' S.L. and 168°30' E.L. at the southeast end. The total distance from Efate to Emirú is about 1550 nautical miles. The Coral Sea and Australia are southwest of this island chain and the Pacific Ocean is north and northeast. In addition, New Caledonia, New Zealand, Fiji, Samoa and other non-malarious islands were used for staging and for rehabilitation of troops from malarious and combat areas.

The major islands of both the New Hebrides and Solomons are of volcanic origin. Coastal areas on many islands are composed of upthrust coral formations which form the subsoil. The topsoil of all coastal plains is alluvial. Green and Emirú Islands are of coral origin. The total area of the New Hebrides is 5,700 square miles; that of the Solomons is 18,000 square miles. The largest island in the New Hebrides, Espiritu Santo, is 76 miles long and 40 miles wide. Efate is 26 miles by 14. The largest island in the Solomons group is Bougainville, with a length of 120 miles and an average width of 40 miles. Guadalcanal, the second largest Solomon Island, is 90 miles in length with an average width of approximately 30 miles. All the islands are covered with dense jungle rain forest or high tropical grass. Coconut plantations are found along the flat coastal plains of many of the islands.

All of the major islands are mountainous. On Bougainville in the Solomons, Mount Balbi reaches to 10,171 feet. Mt. Popomanasni on Guadalcanal is 8,005 feet high and Mt. Tabwemasana on Espiritu Santo reaches 5,940 feet.

Numerous streams are present on the larger islands and are fed by abundant rainfall. They are, in general, short and rapid, although often tending to become sluggish, spreading, and swampy on the level coastal plains and valleys. Mangrove swamps are occasionally found. A high pounding surf on several islands has caused sandbar dams which block streams and produce numerous fresh water lagoons and marginal fresh water swamps.

The temperature throughout the Solomons at sea level varies from a maximum

⁸ Essential Technical Medical Data, South Pacific Base Command, Sept. 1944.

TABLE III
Rainfall (inches)—Solomon Islands

STATION AND YEAR	SOURCE	MONTH												TOTAL
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Tulagi														
1926.....	A	14	13	4	12	5	5	2	9	8	3	13	9	97
1932.....	A	17	10	5	11	8	7	9	13	9	7	19	12	127
1936.....	A	21	14	11	9	10	12	12	4	11	12	5	5	126
Florida (Halavo)														
1943.....	B									6	11	5	18	
1944.....	B	16	15	20	12	8	8	10	13	14				
Guadalcanal														
Lunga Point														
29 yr. pre-war avg.....		13	10	12	7	4	3	3	3	4	4	6	7	76
Henderson Field														
1943.....	B			2	4	9	3			1	3	2	7	
1944.....	B	13	17	12	10	5	5	6	4	5				
Carney Field														
1943.....	B							3	1	2	3	3	11	
1944.....		14	15	11	10	7	5	6	4	4				
Doma Cove														
1944.....	B	15	20	9	8	3		4	3	6				
Russells (Banika)														
1943.....	B					16	4	3	8	3	12	5	3	
1944.....	B	18	19	14	20	12		11	7	7				
Vella Lavella														
Nyanga														
1931-1937 avg.....	C	18	14	15	11	10	8	9	8	7	9	9	8	126
1931-1937 max.....	C	28	21	22	22	13	12	14	13	14	18	13	15	
1931-1937 min.....	C	7	10	7	4	6	5	7	5	5	3	4	5	
Bilca														
1943.....	B										5	4	15	
1944.....	B	19	25	6	10	7								
New Georgia														
Segi													9	
1943.....	B													
1944.....	B	16	8	15	11	9								
Munda														
1943.....	B									10	11	9	14	
1944.....	B	17	16	12	13	12								
Bougainville (Kieta)														
1916-1937 avg.....	D	11	11	11	11	10	9	11	10	8	10	10	9	121
1916-1937 max.....	D	20	36	34	22	16	20	22	20	16	27	16	19	
1916-1937 min.....	D	5	3	4	4	4	4	5	2	2	4	3	1	

A—Pacific Islands Year Book. B—Data collected by U. S. Weather stations and malaria control units. C—Hq USAFISPA Intel. Folder, Ref. No. SPOF-S. D—Hq USAFISPA Intel. Folder, Ref. No. SPOD-9.

of about 95° to a minimum night temperature of approximately 70°. The humidity is high. One can always sleep comfortably cool at night even when

the days are extremely hot and humid. The prevailing wind is from the south-east from April to the beginning of November. From November until the end of March, calms may be expected, with an occasional spell of heavy northwest weather, sometimes continuing for 1 to 3 weeks. Hurricanes do not occur and winds are usually gentle except when accompanied by rain.

Table III presents the available data on rainfall in the Solomons. The rainy season usually begins in November or December and extends through March or April.

The climate of the New Hebrides is similar to that of the Solomons except that temperatures average slightly lower and the annual rainfall is somewhat less. Minimum temperatures are recorded at 53° at Segond Channel on Espiritu Santo and about 58° at Efate, with maximum of 98° and 93° respectively. The prevailing wind, as determined at Segond Channel, is southeast during all months of the

TABLE IV
Rainfall (inches)—New Hebrides

STATION AND YEAR	SOURCE	MONTH												TOTAL
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Tanga I.														
1926-1943 average.....	A	11	10	12	9	8	6	6	6	4	7	8	10	98
Espiritu Santo														
Segond Channel														
1926-1943 average.....	B	15	16	10	10	13	6	5	5	8	6	10	12	116
1943.....	C	7	5	22	9	11	7	3	2	6	20	12	8	112
1944.....	C	13	14	28	19	7	3	8	5	6				
Efate (Vila)														
1943.....	C			13	11	1	3	3	1	5	10	10	7	
1944.....	C	13	13	15	14	5	3	5	3	6				

A—Records of Presbyterian Mission School, Tanga, N. H. B—Monthly Weather Review, Segond, Espiritu Santo, N. H. C—Data collected by U. S. weather stations and malaria control units.

year. The New Hebrides also are classified as an unhealthy area, primarily because of malaria.

Table IV presents data on rainfall. Tanga Island and Espiritu Santo are both in the northern section of the New Hebrides, while Efate is in the southern. Variability of monthly rainfall from season to season and year to year is evident here as in the Solomons, but the rainy season extends from October or November through April or May. Rainfall of 10.5 inches occurred in one 24 hour period, 27 March 1944, on Espiritu Santo.

Guadalcanal and other islands of the southern Solomons were discovered and named by Alvaro de Mendana de Negra in 1567. Bougainville was discovered by the French navigator of that name in 1768.

The Solomon Islands are divided into 2 governmental areas. Bougainville and Buka are part of the Australian Mandate received from the League of Na-

tions in 1920, and are normally administered from Kieta, Bougainville. All the other islands are a British protectorate, proclaimed in 1893, with a British Resident Commissioner at Guadalcanal who is responsible to the British High Commissioner of the Pacific at Fiji.

The principal product of the islands is copra, although small amounts of timber, ivory nuts, trochus shell and green snail shell are also exported. About 5,000 natives are normally employed on the plantations. The value of all exports before the war ranged from 100,000 to 400,000 pounds sterling a year.

The New Hebrides Group was discovered in 1606 by the Spaniard, Pedro Fernandez de Quiros, formerly navigating officer to de Mendana. He was under the impression that he had found the long-sought "southern continent" and he named it *Tierra Australis del Espiritu Santo*, whence came the name of the island of Espiritu Santo. Cook charted the greater part of the group in 1774.

The New Hebrides are governed by a joint British-French Condominium, established in 1906. The British Resident Commissioner is responsible to the High Commissioner at Fiji, and the French Resident Commissioner to the French High Commissioner at Noumea, New Caledonia.

Copra is the chief export, although cocoa and coffee production are also significant. Labor is largely provided by Indo-Chinese (Tonkinese) whom the French brought in. Exports from the islands have been valued at more than 100,000 pounds sterling a year since 1930.

The population of the Solomons, including the Bougainville area, in 1939, was given as 500 whites, 140,000 Melanesians and 200 Chinese. About 20,000 of the Melanesians lived on Guadalcanal, and 45,000 on Bougainville. The indigenous natives of the main Solomons group are Melanesians, although an admixture of Polynesian is evident on certain islands. The peoples of the outlying islands of Ontong Java, Sikiana, Rennell, and Bellona are Polynesian. The natives live rather primitively in small villages or as single families.

Malaria is prevalent among the Europeans and cases of blackwater fever have been reported among them. The Melanesian natives are heavily infected with tuberculosis, filariasis, yaws, and hookworm. Venereal disease is uncommon and syphilis is unknown. Malaria is hyperendemic among the natives. Every person in many localities has had the disease repeatedly before reaching adulthood. The results of pre-war surveys by Sayers and Innes and of numerous surveys by malaria survey groups in 1942-1944 are given in paper IV. The population is said to be declining among those natives who have frequent contacts with the whites and to be increasing among the remaining natives.

The population of the New Hebrides in 1939 was given as 218 British, 687 French, 2,282 Asiatics (Tonkinese, Chinese and Japanese) and about 40,000 Melanesians. Approximately 4,000 natives are thought to be on Espiritu Santo and 1700 on Efate. The indigenous natives of the New Hebrides are Melanesians.

The health status of the New Hebridean natives is generally poorer than that of the Solomon Islanders. The population is steadily declining on almost all

islands. Tuberculosis is common and has a rapid fulminating course among the natives. Yaws, hookworm, the dysenteries, and filariasis also have a considerable incidence. Malaria is hyperendemic among most of the New Hebrides natives.

The initial occupation of the New Hebrides and Solomon Islands by the United States and New Zealand forces can be divided into two phases: the peaceful occupation of the New Hebrides during the first half of 1942, and the occupation by amphibious assault of the Solomons and Bismarck Archipelago bases (August 7, 1942 through March 1944).

The chronological table for occupation of malarious islands is as follows:

a. Efate—advance group landed March 18, 1942. Main landings in April and May, 1942.

b. Espiritu Santo—unopposed landings on May 4 and 28, 1942.

c. Guadalcanal—Amphibious assault on August 7, 1942. Island secured February 9, 1943.

d. Tulagi and adjacent islands—amphibious assault on August 7, 1942. Island secured August 9, 1942.

e. Russell Islands occupied without opposition on February 21, 1943.

f. New Georgia—Amphibious assault (Rendova) on June 30, 1943. Area secured on August 26, 1943.

g. Vella Lavella—Amphibious assault on August 15. Island secured on October 9, 1943.

h. Treasury Islands—Amphibious assault on October 7, 1943. Organized resistance ceased in three days.

i. Empress Augusta Bay, Bougainville—Amphibious assault on November 1, 1943. Perimeter defense until V-J day.

j. Green Island—Amphibious assault on February 15, 1944. Island secured on February 20, 1944.

k. Emiru—Occupied on March 20, 1944.

Enormous changes were brought about by the Japanese and American occupations of these islands. Small perimeters were crowded with thousands of men engaged in diverse activities. Thirty airfields were constructed by Americans throughout the area and approximately eight by the Japanese. Hundreds of miles of all-weather heavy-traffic roads were constructed by the American forces, and thousands of large storehouses, metal huts, hangars, and wood buildings were erected. In many places bitter battles were fought over jungle areas, grass lands and swamps, leaving in their wake a wasteland of shell holes, bomb craters, fallen trees, broken equipment, and other debris of battle. Following the battles, previously uninhabited bays and coves became important harbors, with nearby hospitals, supply bases, staging areas and recreational grounds.

D. AREA ORGANIZATION AND PROCEDURE

The South Pacific Force under Navy Command was a joint U. S. Army, Navy, Marine, and New Zealand group. Commander, South Pacific (ComSoPac) was

the senior Navy Command. The senior Army Command within the area was United States Army Forces in the South Pacific Area (USAFISPA). Island Commanders were responsible to Commander, South Pacific, and Commanding General, USAFISPA. On each base there was an Army Service Command and a Naval Headquarters, each responsible to the Island Commander. Commanding Generals of divisions, if on established bases, were responsible to Island Commanders on matters pertinent to that base.

1. Legal Basis and Development of Area Organization

At the height of the initial malaria epidemic which occurred on Efate, it was requested that an experienced Navy malariologist be sent to Efate to initiate malaria control measures. This officer arrived without a staff or equipment in July 1942. Additional personnel was secured, trained and assigned; directives were issued, and the South Pacific Malaria and Insect Control Organization⁹ herein described was gradually formed.

The slow development of this organization is worthy of comment. It required time to procure and train personnel in the problems of entomology, engineering, and malariology peculiar to this area. Only after field trial was it possible to develop a staff for area headquarters and to make those transfers which were essential to build a strong organization. The directives which formed the legal basis of this organization and established malaria control policy were written and rewritten as new problems were encountered for a period of more than 2 years. The first directive¹⁰ was issued in September 1942 and called attention to the existence of a "malaria control unit," available for use on the 3 bases then occupied, Efate, Espiritu Santo, and Guadalcanal. This directive was issued to publicize the malaria control organization, and in particular to make it available to Espiritu Santo and Guadalcanal, which had been occupied in the preceding weeks. At this time, September 1942, the small number of malaria control personnel in the area was almost entirely Navy, but the mixed service aspect of the organization was foreshadowed by the assumption that this organization would provide malaria control for all services and forces.

Two officers and 8 enlisted men were sent to Espiritu Santo in September 1942 to set up the Base Malaria Control Unit there. Despite the fact that the Guadalcanal malaria rates were rising ominously, no malaria control personnel was allowed to begin operations there until mid-November, when the malaria epidemic was in full swing and anopheline breeding had reached a high level. Such an attitude towards malaria control measures was partly due to the desperate military situation in September and October 1942. However, it was typical of the prevailing opinion that malaria and malaria control were of minor importance during combat operations.

The difficulties in establishing malaria control on Guadalcanal despite the obvious need made it increasingly evident that a stronger area directive was

⁹ See footnote on page 1.

¹⁰ ComSoPac Serial 301c, dated 2 Sept. 1942.

necessary. Such a directive¹¹ was issued in November 1942 and is quoted in part:

"Malaria control units, with headquarters at Base Roses (Efate), have been and are being established at various bases in the South Pacific Area. Each unit consists of a medical officer in charge, an entomologist, and laboratory and field technicians who are specialists in problems of malaria control. These units will advise and render service in connection with malaria control to U. S. Army, Navy and Marine Corps Units and Allied Forces occupying malaria infested islands.

"It is the responsibility of the Malaria Control Units to: (1) make epidemiological studies pertaining to malaria, (2) operate laboratories for diagnosis, (3) train personnel from other organizations in laboratory procedures pertaining to malaria control, (4) advise in regard to mosquito control measures, (5) advise in regard to disinsectization of aircraft, (6) make such recommendations to the proper authorities in regard to malaria control as the circumstances require, (7) procure, store and distribute antimalarial drugs for chemoprophylaxis as may be required by the forces at each base.

"A laboratory section of a Malaria Control Unit will be established at certain non-malarious bases. The officers in charge of these units will carry on studies of malaria infected personnel evacuated from malarious bases and will make recommendations with respect to treatment of and malaria control measures pertaining to evacuated personnel. They will also undertake training of laboratory and medical field technicians attached to organizations preparing to enter malarious bases in the special procedures applicable to malaria control.

"Personnel of Malaria Control Units will be attached to the major medical department activity of the base to which the unit is assigned for administrative purposes, berthing and subsistence. The major medical department activity will also provide laboratory facilities for these units.

"The Commanders of all bases in which Malaria Control Units are established are enjoined to cooperate to the fullest extent with the officer in charge of Malaria Control Units in order that these units may accomplish their extremely important mission. It is directed that officers in charge of malaria control units be consulted in connection with the selection of sites for camps and airfields and that their recommendations in such matters be given due consideration."

This and numerous other directives governing malaria and epidemic control operations were later consolidated into a new directive.¹² There were several significant changes in this new directive. Par. 3b stated that a secondary function of the Malaria Control Organization was to organize and carry on control of epidemic diseases other than malaria. Important excerpts from this directive are quoted:

"ORGANIZATION AND RESPONSIBILITIES

"a. Pertaining to the area program of control. A Malaria and Epidemic Control Officer on the Staff of Commander South Pacific has cognizance of all matters pertaining to the

¹¹ ComSoPac Serial 0024b, November 15, 1942, and a similar directive by USAFISPA published November 29, 1942.

¹² ComSoPac Serial 002263, dated 24 October 1943, General Information Circular, A11-1/MC/(75); and Memorandum 169, Hq USAFISPA dated 19 November 1943.

control of malaria in all forces in the area. He makes recommendations to the Commander South Pacific for the overall area program of control and recommendations for the establishment of Malaria Control Units at bases, and the administration and coordination of malaria and epidemic control. (ComSoPac Serial 0094b, 13 Nov. 1942).

"An Area Entomologist and an Area Engineer serve to coordinate efforts in their special fields. A Training and Education Officer is responsible for an educational program of practical measures of malaria prevention for all shore-based forces in the Area. He prepares such educational material as malaria training manuals for line and medical officers, and for enlisted men. Posters, films and other useful training aids are distributed.

"b. Pertaining to the control program at malarious bases. The senior Malaria and Epidemic Control Officer of base units is directly responsible to the Island Command for an effective program of control, applicable to all forces at the base. He formulates the control program for the base and makes recommendations to the Island Commander who in turn will require subordinate units to carry out prescribed control measures within their respective commands.

"Reports of the senior base Malaria and Epidemic Control Officer are submitted directly to the Island Commander. Copies of such of these as are pertinent are forwarded directly to the senior subordinate commands of the various services at each base, to the Force Medical Officer, Commander South Pacific, the Surgeon, USAFISPA and Headquarters, Malaria and Epidemic Control.

"Base Malaria Control Units are permanently established and serve all forces without service distinction. The units are jointly constituted, being composed of specially trained Army and Navy personnel—malariologists, entomologists, engineers, parasitologists, and laboratory and field enlisted technicians.

"Control operations are carried on under the technical direction of Base Malaria Control Units by the following:

- (1) *Naval construction battalion sanitary sections* consisting of 110 men, together with certain heavy equipment. (ComSoPac 01227, 31 July 1943).
- (2) *Army sanitary companies* performing light engineering duties especially oiling.
- (3) *Native labor*, available at certain of the bases, to carry on clearing operations.
- (4) Mosquito control squads formed within all tactical and service groups.
- (5) Full construction battalions and other units specially designated by Commander South Pacific for malaria control."

The malaria control unit described in this directive, or malaria control group as it was subsequently called, consisted of a malariologist; one or more army malaria survey detachments, comprising an entomologist, a parasitologist and 11 enlisted men; one or more army malaria control detachments comprising a sanitary engineer and 11 enlisted men; or equivalent navy personnel. The command channels of this group are discussed below and are shown in Chart II.

The Area Malaria and Epidemic Control Organization grew and developed as the area expanded from 1 to 11 malarious bases, as the legal basis was broadened and as the personnel increased from 1 officer and 3 enlisted men in July 1942 to over 750 technically trained personnel and nearly 4000 laborers in June 1944. This growth made clear the need for an area staff which was developed in the following order of appointment: An Area Malaria and Epidemic Control Officer; an Area Entomologist; an Army Liaison Officer; an Area Training and Education Officer; an Area Administrative Assistant; an assistant Malaria and Epidemic Control Officer; an Area Engineer and two Filaria Survey Officers.

The duties and responsibilities of the area organization were gradually developed:

a. It served all forces on the basis of its area wide authority and joint U. S. Army-Navy and Allied organization.

b. It formulated area directives which defined and gave authority to insect and rodent control policy, organization and function.

c. It made recommendations for the procurement, assignment and transfer of all control personnel.

d. It provided technical and supervisory assistance to the various base and division malaria and insect control groups.

e. It was responsible for the control work of the base groups and established a uniform system of reports and inspections to allow assumption of this responsibility.

f. It recommended allowances and provided for procurement and distribution of malaria control equipment and supplies.

g. It provided a uniform and area-wide educational and publicity program of malaria and insect control for all personnel, and special information for line officers, medical officers and for personnel assigned to malaria control work.

h. It made personnel and equipment available for special problems of malaria control such as airplane application of DDT solutions and spraying of bed nets with DDT and for problems concerned with filariasis, mite-borne typhus, dengue and rodent control.

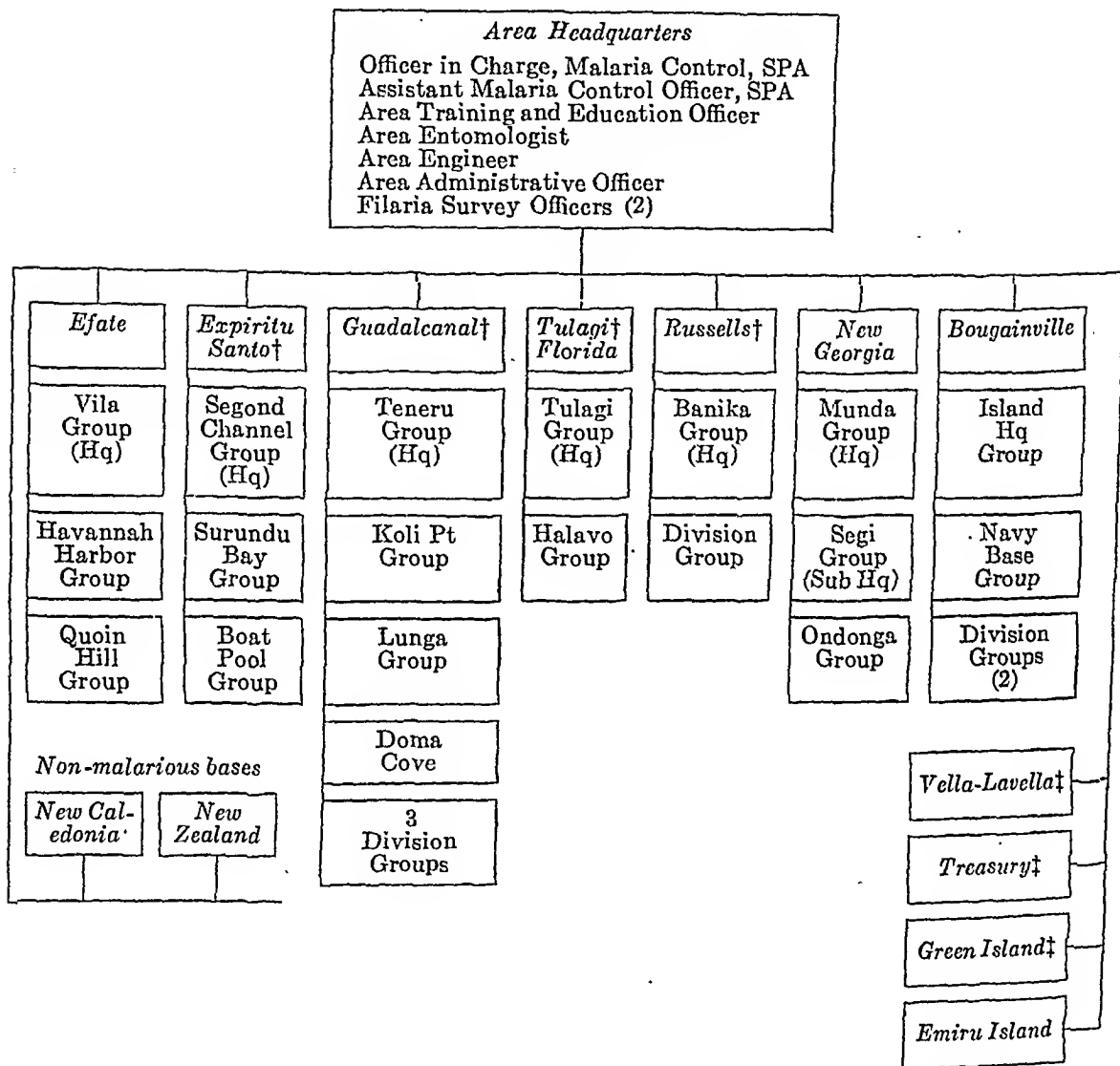
Chart I shows the functional personnel of the Area Organization and the distribution of base and division groups. The members of the area staff had letter orders allowing travel throughout the area. An officer from headquarters made a complete circuit of the bases every 4 to 8 weeks to check on supplies and personnel needs and to discuss current problems. One indication of the enthusiasm and performance of these base units was their pride in their work and their desire that every new project should be visited on these inspection trips. The visiting officer clambered along drainage ditches, slogged through swamps and visited native villages on one base after another until he returned to headquarters, thoroughly exercised and well informed. The area entomologist and engineer often stayed several weeks at a base where current problems required their presence and gave technical and supervisory assistance through personal contact with the corresponding officers in each base unit. Distribution of technical information was also accomplished through the News Letter and special publications such as synoptic keys to mosquitoes. Uniform methods of reporting information were adopted for all base reports as well as for area reports.

2. Extension of Work to Control of Filariasis, Dengue, Tsutsugamushi Disease and Rodent Control

It was discovered that the chief vector of malaria in the South Pacific Area, *Anopheles farauti* Laveran was also the most important vector of filariasis in the Solomon and New Hebrides Islands.¹³ *Anopheles Kolicnsis* was also found to be

¹³ Byrd, E. E. and St. Amant, Studies of Filariasis. Newsletters 14 and 16, August and October, 1944. Headquarters Malaria and Epidemic Control. South Pacific Area.

CHART I. BASE MALARIA AND INSECT CONTROL GROUPS*
Division malaria and insect control groups, South Pacific area (1 June 1944)



* The term Malaria and Insect Control Group is used to designate a working organization comprising a malariologist, one or more army malaria survey detachments and one or more army malaria control detachments or equivalent navy personnel.

† Rodent Control Units were also stationed on these bases.

† Malaria Control Personnel of 3rd New Zealand Division as well as Island Headquarters group.

a vector of Filariasis on Guadalcanal.¹⁴ Thus, the exercise of malaria control on these islands had fortuitously furthered filaria control.

The epidemics of dengue fever on New Caledonia, Efate, Espiritu Santo and

¹⁴ Richer et al. Studies on Vectors of *W. bancrofti*, Newsletter No. 26, July 1945 and ComSoPac Occasional Papers No. 1, Sept. 1945 and No. 2 Oct. 1945, Headquarters Malaria and Epidemic Control, South Pacific Area.

Tualagi-Florida are noted above. Local malaria control groups had given repeated warnings of the potential hazard of tin can dumps and other breeding places of the dengue transmitting mosquito, *Aedes aegypti* (L). With the outbreak of these epidemics, the organization was given adequate authority, personnel and equipment to cope with the problem. As a result there was no dengue outbreak in 1944 on any base except New Caledonia where it was held to small proportions.

The outbreak of tsutsugamushi disease (mite-borne typhus) on Bougainville led the local malaria control organization to assume responsibility for protective measures including impregnation of clothing with dimethylphthalate. Study of this disease and its control was aided by the arrival of an advance unit from Naval Medical Research Unit No. 2 (11) which was attached to the Base Malaria and Insect Control Group.

Rodent Control was undertaken both to prevent economic loss from rats and to prevent the spread of epidemic diseases which were harbored or transmitted by rats or their ectoparasites. An officer qualified as a mammalogist or with civilian experience in rodent extermination was appointed as rodent control officer and attached to the malaria and insect control group at each large base. An adequate number of enlisted men, equipment and transportation were provided. A manual was prepared and men from each military unit on the island were trained in the technique of rodent extermination and their work was then supervised. Fumigation of ships for rats was done on request.

S. Procurement and Assignment of Personnel

Table V summarizes the sources and number of personnel available to the South Pacific Malaria and Insect Control Organization as of 15 May 1944. The technically trained personnel comprised medical officers, entomologists, parasitologists, sanitary engineers, and enlisted personnel of the control and survey detachments. They formed the Base and Division Malaria and Insect Control Groups and were the technical and administrative nucleus of the entire organization. As of May 15, 1944, this personnel comprised 128 officers and 643 enlisted men, divided as follows: Army—452; Navy and Marine—282; New Zealand—37.

a. Technically trained army malaria control personnel was provided by the War Department in 3 categories: Malariologists (medical officers), Malaria Survey Detachments and Malaria Control Detachments.

Army malariologists arrived as casual officers. Almost all of these officers had taken the course in Tropical Medicine at the Army Medical School and the majority had had field work at the army school in Florida or Panamá. They were attached to Headquarters, Service of Supply, South Pacific Area, and then ordered on detached service to the various bases and divisions. There were 21 army malariologists in the area; 2 Lt. Colonels, 8 Majors, and the remainder Captains. One army malariologist was on the Area Malaria Control Staff, 6 were senior base malariologists, 6 were division malariologists, 1 was assigned to the Office of the Chief Surgeon, USAFISPA, 1 to Headquarters XIV Corps, and the remainder acted as assistant base malariologists.

Army Malaria Survey Detachments consisted of an entomologist, a parasitologist and 11 enlisted men, with 5 vehicles¹⁵ and adequate laboratory equipment for all ordinary entomological and parasitological survey work. Army Malaria Control Units consisted of a sanitary engineer and 11 enlisted men and were authorized 8 vehicles and light engineering equipment.¹⁵ Officers were well qualified, and enlisted men were usually of high calibre and rapidly became competent.

The army control and survey detachments were small commands and were transferred or assigned intact. There were 17 Malaria Survey Detachments and 20 Malaria Control Detachments within the area, as of 1 June 1944. One addi-

TABLE V
Malaria and insect control personnel—SPA
15 May, 1944

DESCRIPTION	SOURCE	NUMBER
Technically Trained Personnel* Malariologists, Entomologists, Parasitologists, Sanitary Engineers, Rodent Control Officers and Trained Enlisted Personnel	War and Navy Depts. Special Survey and Control Detachments for Tropical Disease	771
Skilled Labor Operators of Heavy Equipment Dynamite crews Flume crews, etc.	Navy Construction Battalions Army Engineer Corps	634†
Unskilled Labor Medical Sanitary Companies Troop Antimalaria Details Natives	War Department 936 Troop Units 1479 Local Government Agency 587	3002
Total.....		4407

* Base and Division Malaria and Insect Control Groups.

† Average figure for 6 months, December 1943-June 1944.

tional survey unit and 2 control units had been trained and staged for the South West Pacific Area.

b. Navy malaria control personnel was procured through the Navy Department either from the Malariology School of the Naval Medical School, Bethesda, Maryland, or from Navy replacement pools or other organizations within the South Pacific. The usual Navy malaria control team consisted of one officer, an entomologist, and 3 to 5 enlisted men. Larger teams of 3 officers (malariologist, entomologist, parasitologist) and 12 enlisted men were supplied to Marine Divisions. An engineer for this group was provided with the sanitary section of the attached construction battalion, see below. Enlisted men from Bethesda were well trained in laboratory diagnosis and in elementary field procedure.

¹⁵ TO/& E No. 8-500, 13 May 1944.

Several Navy officers experienced in rodent control were obtained from organizations in the South Pacific. Navy warrant and Hospital Corps officers were also secured locally to administer malaria control personnel and supplies on larger bases.

The size of a base unit was established by local requirements, and no predetermined limitation was set. For this reason Navy malariology teams were broken up freely and fitted to existing needs for expansion or replacement in mixed Army-Navy or all-Navy groups.

c. Skilled Labor and Heavy Equipment. Skilled labor for malaria control work included dragline and bulldozer operators, dynamite experts, carpenters, and welders to make flumes and culverts. Such skilled personnel and heavy equipment were obtained chiefly from Naval Construction Battalion personnel, and in small part, from the Army Corps of Engineers.

The formation of Sanitary Sections in all Naval Construction Battalions for use on malaria control work was authorized by a series of directives.¹⁶ These directives ordered formation in each Naval Construction Battalion of a sanitary section of 110 enlisted men plus a specified list of equipment for work on malaria and epidemic control projects under the direction of Base and Division (Marine) malariologists. Equipment assigned to each sanitary section included 1 dragline crane, 1 tractor with bulldozer blade, and 7 trucks. These directives made available to malaria control a potential total force of over 2000 men and more than 20 bulldozers, 20 dragline cranes and 140 vehicles from the 20 or more Naval Construction Battalions that were on malarious bases. Actual compliance with these directives furnished about 500 SeaBees, 10-15 bulldozers, and 8-12 dragline cranes daily for work on malaria control projects during the 8 month period, November 1943 to June 1944.

Despite outstanding work by many of these battalions, compliance with these directives was usually delayed and incomplete. Work often was done too late to forestall an initial outbreak of malaria and seeding of troops. This delay was due to high priorities for airfields, roads, harbor and storage facilities. Requests for diversion to malaria control of 10 per cent of men and equipment often seemed unreasonable to the officers responsible for major construction projects. This early attitude was fostered by the routine use of suppressive atabrine which temporarily hid the full extent of seeding with malaria. Furthermore, certain faults were inherent in the sanitary section of the construction battalion as originally conceived. Construction battalion personnel comprised for the most part rated men and highly skilled labor, the number of seamen decreasing with every month overseas. It was wasteful and damaging to morale to use skilled labor for unskilled manual work. Wherever possible the use of skilled construction battalion labor was limited to the use of heavy equipment and to other skilled jobs while unskilled manual work was done by native labor.

¹⁶ a. VCNO ltr. OP 30 Pz-MP (SC) P 2-3, serial 0613830, dated 9 July 1943;

b. BuDock Directive, PacDiv Serial 948, YDI-me, dated 17 May 1943;

c. ComSoPac Serial 01227, dated 31 July 1943; and

d. ComSeronSoPac Serial 0744B, dated 6 August 1943.

In May 1943, before receipt of the above directives, the need for large scale mosquito control work on Guadalcanal became so urgent that the entire 63rd Naval Construction Battalion was ordered by ComSoPac to malaria control work at that base. The personnel of this battalion rapidly became acquainted with malaria control problems and techniques and accomplished an extraordinary amount of semi-permanent control work over the entire base.

The use of Army Engineers for Malaria Control was authorized¹⁷ as follows: "The Corps of Engineers is charged with the responsibility for the execution of mosquito control work on real property. This includes such measures as drainage, filling, larvicidal programs and screening." The number of Army Engineers Corps troops in this area was small, as compared with Naval Construction Battalions. The use of such troops for malaria control projects was subject to the same delays encountered with Naval Construction Battalion Sanitary Sections with the added handicap that no set per cent of Army Engineer troops was directed to do malaria control work. A few Engineer Corps troops did excellent work on insect control projects but the total was small.

The practice begun during 1944 of submitting consolidated estimates for all base malaria control projects to the Commanding General, with the request that these projects be assigned to heavy equipment units, resulted in division of these projects between Army Engineer Units and Navy Construction Battalions. Such projects were well prosecuted.

In summary, the malaria control organization rarely succeeded in so presenting the need for heavy equipment for mosquito control as to secure its accomplishment in the early days on a base. Eventually, an adequate amount of skilled labor and heavy equipment was made available on all bases, but only after delays which contributed to seeding of troops with malaria and dengue and to great loss of man days and efficiency. On most bases such work was delayed more than 6 months after occupation. Only on Emiru Island in the St. Matthias Group, the last operation in this area, was a significant amount of heavy equipment made available for mosquito control work within 3 months of occupation.

A large share of semi-permanent work was done with borrowed equipment which was operated by personnel of malaria control detachments and medical sanitary companies. Malaria Control personnel on Guadalcanal operated an average of 10 bulldozers, 2 draglines and several disc harrows throughout 1944, and similar personnel on Santos and Bougainville operated about half this amount of equipment. These experiences led to a recommendation to the War Department, approved by Headquarters, Services of Supply, South Pacific Area, to add such earth moving equipment to the table of equipment of medical sanitary companies. Only by some such plan would it have been feasible to accomplish semi-permanent control work during the early months on a new base.

d. Unskilled Labor. Unskilled labor was used for oiling, for hand clearing and ditching, and for other details. It was obtained from 3 sources: army medical sanitary companies, troop unit antimalaria details and natives.

¹⁷ AR 100-80, as quoted in par. 2, WD Circular No. 223, 21 September 1943.

Each Army Medical Sanitary Company consisted of 3 white officers and 109 colored enlisted personnel. (One Sanitary Company had colored officers.) These companies provided their own messing facilities and were authorized¹⁸ 9 vehicles and other suitable equipment. They were assigned to Island Commands and were employed as directed by the malariologist in conjunction with antimalaria work. There were 8 Medical Sanitary Companies used only for malaria control within the South Pacific Area as of 1 June 1944, located as follows: 4 companies on Guadalcanal; 1 each on Russell Islands, Munda, and Bougainville; and 1 divided company with a platoon at Green Island and a platoon at Emirau Island. They rapidly developed an understanding of the problems of malaria control and facility in necessary procedures. Enlisted men who showed aptitude were trained in the operation and maintenance of heavy equipment, in dynamite work and in mosquito survey work. These troops were of great value as a constant source of experienced labor.

Troop unit antimalaria details¹⁹ were charged with oiling, minor drainage work, and other control measures in their bivouac area. These details consisted of 3 men from each company or similar unit and were the backbone of all insect control work.

Natives were employed on nearly all bases. The decision to utilize this source of labor was made early in the campaign with the knowledge that natives constituted a potential seed bed of malaria and filariasis. The malaria control organization attempted to minimize this health hazard by segregation of natives and by other means described under section on malaria control measures. The Melanesian natives in the New Hebrides were controlled by the Condominium Civil Government; those in the Solomon Islands by the British Solomon Islands Protectorate. Natives were recruited from outlying islands or areas under Colonial government supervision and allotted to the various military bases. Only healthy adult males (except at Bougainville) were accepted. A fixed contract as to period of hire, wages, food, quarters, and hours of work was established by the Colonial government. Native laborers in the Solomons were formed into semi-military organizations under white officers. This labor was paid by the government of the British Solomon Islands Protectorate and furnished to U. S. Forces without charge as one of their contributions to the war effort. It was agreed that the U. S. Government would provide such native labor with quarters, rations and certain other benefits and that these supplies would not be charged against the British Solomon Islands Protectorate under lend-lease. On most islands the natives were under the immediate supervision of an Australian, New Zealand or other labor corps officer. Natives worked in sections of 25, each with its own native sergeant. Transportation to and from work was provided by the military activity using them. The total number of imported Melanesian laborers on all bases was over 6000 in 1944. About 600 or 10 per cent of these natives worked daily on malaria control during the period of maximum activity in the theatre.

¹⁸ T/O & E No. S-117, 13 May 1944.

¹⁹ See par. E4 below.

4. Supplies and Equipment

There were acute shortages of all antimalaria supplies and equipment in 1942, of which the most important were atabrine, mosquito repellent, insecticide and knapsack sprayers. In this early period the area malaria control organization advised on allowances and was responsible for the establishment of quotas and distribution of those items in which shortages were acute. Excerpts from the pertinent directive follow:

HEADQUARTERS SERVICES OF SUPPLY

AG 729.5 (5-11-43)

APO 502

13 May 1943

Circular

ISSUE OF INSECTICIDE

No. 15

PEST CONTROL SUPPLIES AND EQUIPMENT

"1. Control, procurement, distribution and issue of insecticides, pest control supplies and equipment for all Armed Forces located in the South Pacific Area has been assigned to the Army Services of Supply by ComSoPac.

"2. Service Command Quartermasters . . . in cooperation with the Base Malaria Control Officers, will receive, store, and distribute the above supplies to all Armed Forces at each base. Navy Supply Officers, Marine Quartermasters and New Zealand Supply Officers may obtain their stocks in bulk from Base or issue Service Command Quartermasters at each Base, by requisition.

"5. The use of insecticide and insect repellents within the South Pacific Area will be governed by instructions issued by each base malaria control officer."

The inclosures to this circular fixed allotments for pest control supplies and equipment. Supplies were distributed to Island bases by automatic issue as they became available. This arrangement worked well. At first it was necessary to lower the quotas of certain critical items below the allowances given in Circular 15. The policy was to curtail all rear base quotas in an effort to assure adequate supplies to the forward and combat bases. The supply of freon aerosol dispensers is an example. This item became available in significant quantities about September 1943. The area malaria control staff recommended that the quota for troops in the Southern Solomons be fixed at 100 dispensers per 1000 men per month and that only troops moving to combat areas in the Northern Solomons receive a full allowance, which at that time was 225 dispensers per 1000 men per month. Supplies of all items except DDT were adequate by March 1944 and no further quota restrictions were necessary.

Acute area shortages of atabrine both for treatment and suppression were encountered during 1942. Early in 1943 an adequate quantity of atabrine was available and a system of priorities was no longer necessary. Quinine was used extensively for suppression and clinical treatment during 1942 and early 1943, but its use waned as the value of atabrine was demonstrated.

Transportation was often a serious problem. Each Malaria Control Group was responsible for a territorial coverage of about 20 square miles, often in the shape of a long narrow beachhead. In addition to distributing their own survey and control crews to all parts of this territory, most groups transported 50

to 100 native laborers to and from work each day and hauled labor details. They also did power spraying, hauled gravel and did other work requiring vehicles. The increased number of vehicles which were finally authorized for malaria detachments and for medical sanitary companies were adequate. A special directive provided transportation for malariologists.

The majority of all personnel lived in pyramidal tents; others in prefabricated huts. Buildings were required for offices, laboratories, classrooms, store houses and workshops. Quonset huts and other material for these buildings were supplied in 1943 through Navy channels. Subsequently the Island Command at most bases provided additional housing.

E. BASE OR ISLAND ORGANIZATION AND PROCEDURE

1. The Work of the Malariologist

The legal basis for the work of the island malariologist has been described.²⁰ By these directives the senior malariologist at each base eventually was made directly responsible to the Island Commander for the formulation of a program of control applicable to all forces, Army, Navy, Marine and Allied, and for recommendations to make this program effective. The commanding officer of each subordinate organization was responsible for all malaria control activities within and adjacent to his bivouac site. Reports and recommendations of the senior base malariologist were submitted directly to the Island Commander, who, in turn, required the subordinate commander to carry out prescribed control measures. This chain of command was unusual in that it did not conform with the ordinary channels through the Commanding Officer of Service Command or through the Commander, Advance Naval Base. The proper choice, of channel was uncertain with a joint Army-Navy malaria control organization which might be headed by an Army or a Navy malariologist or by both at successive periods. This joint organization, moreover, supervised malaria control projects which involved both services and made inspections and reports on the malaria discipline of all forces. Direct access to the Island Command was authorized to obviate these difficulties and because the Island Command had a greater ultimate responsibility to reduce the island malaria rate than had any subordinate command.

The organization of the Army-Navy malaria control personnel on each Base is also shown by Chart II. A mixed Army-Navy group was developed on most islands. The variable size of the Navy units was particularly advantageous for small bases where only one or two officers and a few enlisted men were needed and where the army units of fixed size were too large. The service distribution of the technically trained personnel is given in Table VI, and of the labor in Table IX. The efficiency and economy of this joint use of personnel provides a stimulating chapter in combined service organization.

Chart III, Guadalcanal Malaria Control Organization, is presented as an example of organizational development on islands large enough to require 2 or

²⁰ ComSoPac 002263 and Memo Hq. USAFISPA quoted in Sec. D.1.

CHART II. BASE ORGANIZATION AND COMMAND CHANNELS
Joint Army-Navy malaria and insect control

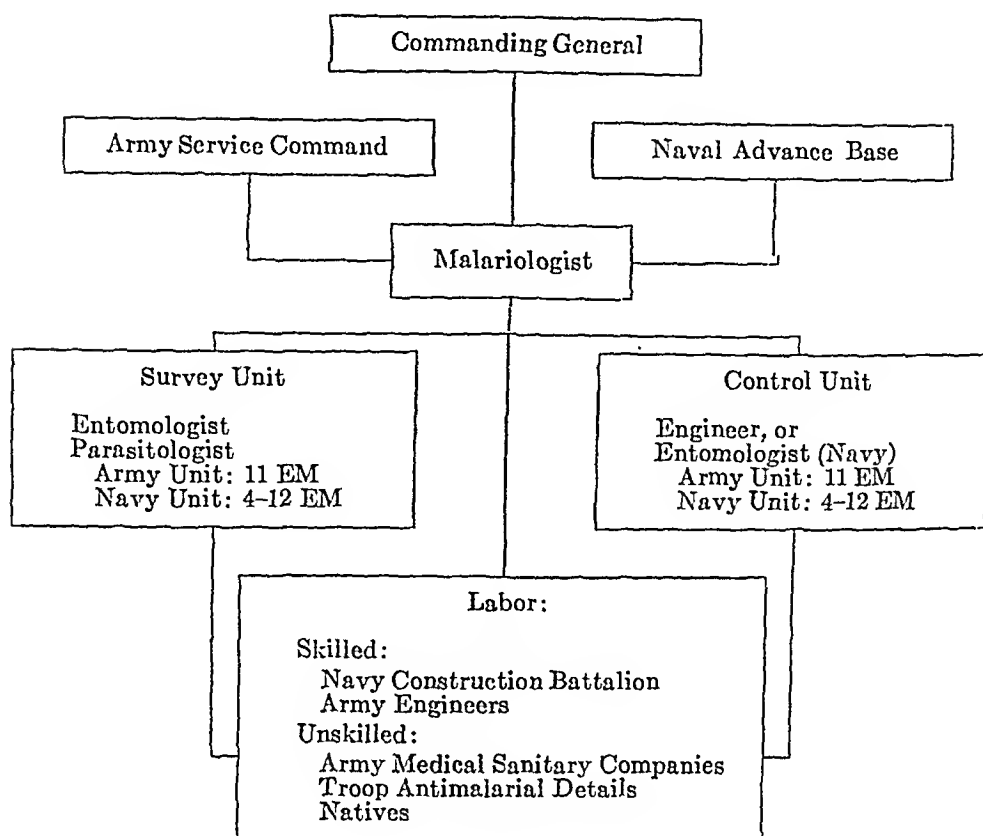


TABLE VI
Base malaria control personnel, South Pacific area
(As of 15 May 1944)

BASE	ARMY		NAVY		TOTAL
	Officers	Enlisted	Officers	Enlisted	
Hq. MalConSoPac.....	2	1	3	3	9
New Zealand.....	1	0	0	4	5
New Caledonia.....	7	47	3	14	71*
Efate.....	4	22	2	11	39
Espiritu Santo.....	2	11	17	40	70†
Guadalcanal.....	31	179	2	20	232‡
Tulagi-Florida.....	0	0	5	25	30
Russell Islands.....	0	0	5	28	33
Segi, New Georgia.....	0	0	1	4	5
Munda.....	5	22	3	12	42
Vella Lavella.....	0	0	1	14	15
Treasury.....	0	0	2	10	12
Bougainville.....	6	33	1	8	48
Green Island.....	1	0	2	14	17
Emiru.....	4	22	2	10	38
Total.....	63	337	49	217	666

* Includes 50 staging personnel (Army).

† Includes staging personnel (Navy).

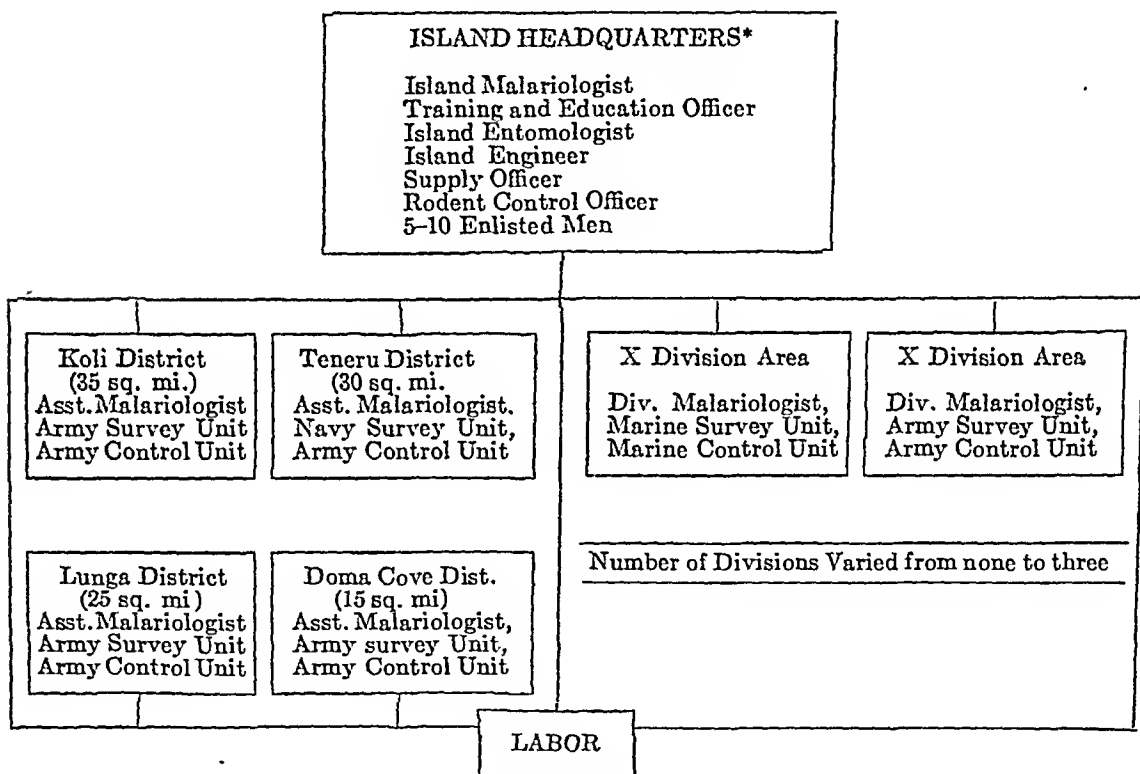
‡ Includes 113 staging personnel (Army).

Note: This table comprises technically trained personnel only. Divisional personnel are omitted. See Table IX for distribution of labor personnel from various services.

more malaria control groups.²¹ The Island headquarters was not provided for by any Table of Organization but developed to fill a need for overall supervision on larger bases. Modifications of the headquarters section, diagrammed in Chart III, were established on Efate, Espiritu Santo, New Georgia, and Bougainville.

The malaria control personnel on most bases formed a joint Army-Navy group who lived under one roof and worked together in one area. Two-thirds of the groups were attached to Army or Navy hospitals for rations, one-third to

CHART III. GUADALCANAL MALARIA AND INSECT CONTROL ORGANIZATION



* No authorized table of organization for this headquarters. Supply Officer and Rodent Control Officer are Navy personnel, others are Army.

Navy Advanced Bases or Army Service Commands. Three groups set up independent housekeeping with a Medical Sanitary Company assigned to malaria control work. This had distinct advantages, among which was a time for messing which was most advantageous for field work.

The position of the malariologist in this organization was an ambiguous one which can be explained best by saying that he was a staff officer whose duties often required the assumption of command responsibility. This was evident in

²¹ The term Malaria Control Group is used to designate a working organization comprising a malariologist, a malaria survey detachment and malaria control detachment.

his relations with assistant malariologists, and with personnel of survey detachments, control detachments and sanitary companies. He selected their locations, directed their work and initiated or approved recommendations for promotion. This assumption of command responsibility functioned well because of a general willingness to cooperate and because the high command fostered such a situation by directives quoted above which made the malariologist responsible for all insect control activities on a base. However, the malariologist had no legal command authority over the malaria detachments and medical sanitary companies which were small independent commands. In a long range program this officer should have command authority corresponding to his responsibility.

The duties of the malariologist included:

- a. Planning an effective program of mosquito control utilizing the advice and assistance of specialists, the entomologist, the parasitologists and the engineer.
- b. Integration of the work of the survey detachment, the control detachment and labor.
- c. Estimation of need for and requisition of personnel and supplies to execute his program.
- d. Development of an effective malaria training and educational program (Sec. F.).
- e. Preparation of directives pertaining to malaria discipline and the making of spot inspections for violations of malaria discipline.
- f. Consultation and recommendation in regard to the selection of sites for camps, airfields, bivouacs and maneuver areas.
- g. Segregation of natives.
- h. Recommendations concerned with the institution and discontinuance of suppressive medication.²²
- i. Supervision of disinsectization of airplanes and ships and other measures to prevent the dissemination of disease.
- j. Preparation of reports of the malaria situation on his base or in his division area, including especially statistics regarding malaria incidence, status of malaria discipline, entomological and climatological data, work of control units, activities of the training program, status of anti-malarial supplies and of personnel engaged in control work, recommendations.

The malariologist, in execution of these duties, provided frequent, up-to-date estimates of the insect-borne disease situation on his base or in his division area. This information was obtained prior to the occupancy of a new base from the literature, from colonials with pre-war knowledge of the area, and from data gathered by Army and Navy intelligence sections. The study of rainfall figures and aerial mosaics was valuable in estimating probable breeding sites. More reliable information was obtained after occupancy of an island from the entomologist's surveys of mosquito populations, from data of malaria incidence in troops and natives, and from the engineer's reports of existing control activities

²² ComSoPac Serial 02259 Nov. 1944.

and future requirements. He formulated an effective control program based on these reports, the advice of his specialists, and his knowledge of the tactical and supply situation. The first work was done where troops were concentrated. Small, isolated outposts were furnished with sprayers and other supplies but were required to do their own antimalaria work during this early period. Speed in instituting control measures was most important in occupying a new base. Initial surveys were done rapidly and more thorough work came later. A larvicidal program and other temporary work, such as clearing of paths to facilitate oiling, was usually initiated coincidentally with the first survey.

The initiation of semi-permanent work depended on the size of troop population to be protected, the period the area was to be occupied, and on available labor and equipment. As soon as surveys were completed a list was prepared of semi-permanent control projects with detailed estimates of labor and equipment, see Paper III. These projects were listed in order of priority and were initiated directly if they were within the scope of the malaria control personnel under the jurisdiction of the malariologist. Larger projects requiring special equipment and labor were submitted through proper channels to the Commanding General for approval and for assignment of the needed equipment and personnel. These projects competed with other high priority work such as road building, airfield construction, and erection of hospitals. Presentation of a project had to be clear, concise, specific and had to include an adequate justification for priority.

The need for continuous integration of the work of the survey and of the control units was recognized rapidly. Apparently the original plan envisaged a survey unit that would land in the early days of occupancy of a base, would determine the problem and lay down a plan of operation for the control unit which would arrive at a later date. In war time practice, survey and control work were initiated simultaneously and continued to be interdependent. It was a part of the early experience of newly arrived entomologists that survey work was of immediate military value to the extent that it was translated into control of insects. Likewise it was the daily experience of engineers that their efforts to control insect breeding were much more effective if closely correlated with the work of the entomologist and the parasitologist. This team work between survey and control personnel was achieved by the joint use of living quarters, office and laboratory space and particularly by the efforts of entomologists and engineers in their daily field work, see Sec. D 3 of Paper III. The malariologist was in constant touch with these units and with his labor groups to assist in the solution of their supply and personnel problems. The effective integration of the work of these various units was one of the chief measures of his success.

Supply problems and many minor administrative problems often required much of the time of the malariologist. On the larger bases this work frequently was delegated to Navy warrant officers who were assigned for that purpose. Storehouses (fig. 1) were built and inventories of anti-malaria supplies in the quartermaster, ordnance and engineer dumps were maintained. Guadalcanal

became a supply base for all malaria and insect control groups in the Northern Solomons.

Further discussion of the duties of the malariologist are found in other sections, i.e., Sec. F. The Training and Education Program.



FIG. 1. CORNER OF STOREHOUSE FOR INSECT CONTROL SUPPLIES ON GUADALCANAL

TABLE VII

Entomological section of survey detachment—example of personnel organization

DESIGNATION	NUMBER	DUTIES
Officer	1	Entomologist in charge of entomological activities
Senior N C O	1	Direct supervision of both field and Laboratory work
Laboratory men	2-3	Map making, mosquito population records, rainfall records, care and identification of specimens brought to and reared in insectary
Field men	5-8	Field men survey 3-5 square miles per man. A 5 man crew covers an area of 15-25 square miles

2. The Malaria Survey Detachment

The malaria survey detachment consisted of two officers and eleven enlisted men, all technically skilled, and charged with entomological and parasitological work to aid the control of malaria and all other insect borne diseases. Table VII shows the organization and duties of the entomological section of the survey detachment.

The entomologist and his enlisted men furnished information about the breeding of mosquitoes and other insects, their biology and relations to disease. This information was always accompanied by recommendations as to specific control measures. This work was continuous and was recorded on maps and other forms so as to give a clear and continuous check on the effectiveness of control. The preparation of adequate maps from aerial mosaics and ground inspection was fundamental to insect control work and was one of the responsibilities assumed by the entomologist.

The parasitological section of the survey unit comprised 1 officer, a parasitologist, 1 senior non-commissioned officer in charge of the parasitology laboratory and 1 to 3 enlisted laboratory technicians who provided information about parasitic diseases of military importance. They furnished information about the incidence of malaria and other parasites in natives, in our own troops and

TABLE VIII
Malaria control detachment—example of personnel organization

DESIGNATION	NUMBER	DUTIES
Officer	1	Engineer, SnC., or II-V (S) in charge of all control activities
Senior NCO	1	General supervision, asst. to Engineer
NCO	1	Contact with troop oiling squads and work details from Sanitary Company
NCO	1	Contact with work crews of heavy equipment units; with level and transit crews
NCO	1	Clerk
NCO	2	Supervisors of native crew
NCO	1	Utility repair man to service hand sprayers, power sprayers and dusters for all organizations
NCO	1	Vehicle Dispatcher and repair man
Pfc or HA/2c	1	Truckdriver, dump truck
Pfc or HA/2c	1	Truckdriver, powersprayer
Pfc or HA/2c	1	Truckdriver, general

Japanese prisoners and recorded this knowledge so as to aid both the planning and the evaluation of control work.

Both the entomological and parasitological sections participated actively in the training and education program and in other aspects of the work. Details of the entomological and parasitological problems and methods in the South Pacific Area are presented in papers III and IV.

3. The Malaria Control Detachment

The name of this unit, like that of the survey detachment was a misnomer because its work embraced the planning and execution of all control activities directed against not only malaria, but also dengue, filariasis, tsutsugamushi disease, fly borne diseases and occasionally against pests. After moving from the South Pacific to other areas many of these units were used in the control of Japanese B encephalitis, schistosomiasis and other diseases. The usual organization of a malaria control detachment is shown in Table VIII.

The commanding officer of a control detachment was an engineer and was responsible for planning, execution and maintenance of all insect control measures based on the findings of the survey detachment; for supervision and correlation of all labor and equipment for this work; and for maintenance of suitable records to give a continuous and clear picture of control activities.

The enlisted personnel performed a variety of duties, according to the local situation. These men were most economically and efficiently employed as supervisors. Occasionally, on large bases, an entire control team was made responsible for a special project such as work on flume and culvert maintenance. Additional enlisted personnel were assigned to work with dynamite or bangalore torpedo

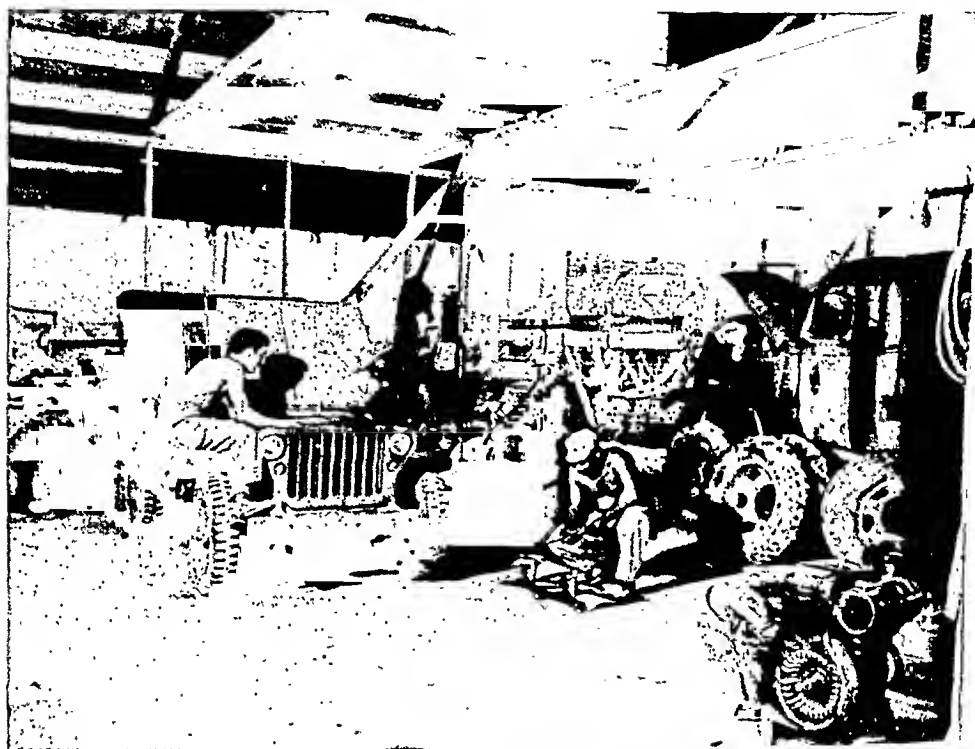


FIG. 2. MOTOR REPAIR SHOPS OF THIS TYPE WERE OPERATED BY MALARIA CONTROL HEADQUARTERS ON LARGER BASES

ditching crews. Men were trained to run bulldozers, operate draglines, and other heavy equipment. On islands where airplane spraying of DDT solutions became an important control measure, crews of 1 to 6 men were assigned to mix DDT solutions and to service the spray apparatus installed in the planes.

A motor repair shop (fig. 2) was organized on many islands in order to avoid delay in obtaining service at regular army motor repair units. Thus, on Guadalcanal where the malaria control organization had over 100 vehicles to keep in service, a repair shop was staffed by mechanics in the local organization and was equipped to do all types of motor repair work except rebuilding jobs. A welding shop was established for making flumes and for repair work.

Table IX shows the average number of laborers available for the work of the

malaria control units during the period of 6 months from December 1943 to June 1944. The engineer was responsible for the work of these laborers except that of the troop anti-malaria details. Thus, on the larger bases, each engineer had from 100 to 250 men, exclusive of troop units, working under his general supervision. There were 4 engineers on Guadalcanal, so the figure for that base was divisible by 4 to obtain the labor available to each engineer.

4. Troop Unit Anti-Malaria Organization

From the first, antimalaria details were expected to do the larvicidal work in their own areas. The assignment of men to oiling details in 1942 and the early half of 1943 was irregular and depended on personal arrangements between the

TABLE IX
*Labor available for work of control unit**

BASE	AREA TO BE CONTROLLED	NUMBER OF LABORERS					
		Base control unit	Seabee or Army Eng. unit	Med. San. Co.	Natives	Troop antimalaria details	Total
	<i>sq. miles</i>						
Efate.....	39	15	12	0	95	40	162
Espiritu Santo.....	40	33	10	0	0	350	393
Guadalcanal†.....	110	55	355	202	250	350	1302
Tulagi-Florida.....	9	18	35	0	37	70	160
Russells.....	15	15	42	117	40	150	364
Munda.....	20	18	35	117	75	80	325
Bougainville†.....	50‡	22	10	117	80	150	379
Green Island.....	15	5	12	65	0	125	207
Emiru.....	30	15	60	50	0	60	185
Vella Lavella.....	20	10	33	0	10	?	53
Treasury†.....	7	4	30	0	0	104	138
Total.....		210	634	758	587	1479	3668

* Average figures for six months, December 1943 to June 1944 (except Emiru which was occupied in March 1944).

† Omits divisional units.

‡ Controlled area increased from about 30 to 50 square miles during above period.

island malariologist and each commander. In February 1943 the Commanding General on Efate ordered the formation of an anti-malaria detail in each company or similar unit. In April 1943 a division commander ordered approximately 1 per cent of his command to fulltime malaria control work. In May 1943 the Commanding General on Guadalcanal ordered that 2 per cent of the command strength be diverted to malaria control activities. In July 1943 a malaria control group was attached to Corps Headquarters for the initial landings in the New Georgia campaign. Specially qualified medical department personnel, hereafter described as Malaria and Insect Control Groups, were attached to Marine divisions in July 1943 and to Army divisions²³ in September 1943. In

²³ AG 370.5 Hq USAFISPA, 29 September 1943.

September 1943 an area wide directive²⁴ ordered the formation of a mosquito control squad in every battalion. These were later consolidated in a single directive²⁵ which ordered the formation of an antimalaria detail in each company, battery or similar unit. This detail consisted of 1 non-commissioned officer and 2 enlisted men per infantry company or a proportionate number for other units. In non-medical units these details were made up of non-medical personnel. These details were responsible for all insect control work within the region occupied by their units and their work was checked by technicians from the base or division malaria survey detachment described above.

These anti-malaria details worked effectively in all situations except those of front line combat. It was not only impossible for most anti-malaria details to do antimosquito work under combat conditions, but this personnel was as fatigued as their comrades at the end of the combat period and so further postponed this work. To remedy this situation, temporary spray teams were formed in combat regiments and are described below.

5. Division Malaria and Insect Control Organization

The Division Malaria and Insect Control Group comprised the division malariologist, a malaria survey detachment and a malaria control detachment totaling 4 officers and 22 technically trained enlisted men. This group was attached to the division over and above its established allowance for medical department personnel. They performed the same functions for the division as did the base malaria and insect control group for each island base. The detailed duties and responsibilities of the malariologist, the entomologist, the engineer, and the parasitologist were similar to those outlined above, with the additional duty of providing anti-malaria protection during the periods of active combat. This resulted in less emphasis on specialization and more emphasis on flexibility, with every man trained to aid in all phases of a simple antimosquito program.

It was the duty of the division malariologist to provide plans for the control of malaria, dengue, mite-borne typhus, and other insect-borne diseases during a period of active operation. Several plans were prepared contingent on such factors as advance information of medical problems, the anticipated speed of the operation and whether the division was to act as a compact unit or was to be spread out over a wide front as separate regiments or battalions. The final plan, selected from several prepared in advance was determined by the particular military situation.

An important feature of all such plans was the provision for a pool of trained men to do temporary insect control work behind the lines during combat periods. This pool of personnel was obtained by drawing 1 man from each antimalaria detail and adding a technically trained nucleus from the attached survey and control detachments. In most plans this personnel was split into 4 temporary spray teams, 1 team attached to Division Headquarters and 1 team to each of the 3 regimental Headquarters. These temporary spray teams gradually im-

²⁴ ComSoPac serial 01619, dated 13 September 1943.

²⁵ ComSoPac Serial 02158, dated 19 October 1944.

proved in operation and functioned well for combat units which staged in the South Pacific for the Peleliu and for the Okinawa campaigns. The temporary spray team went ashore with the division or regimental headquarters to which it was attached and began work. Fly control was done by spraying dead bodies with 5 per cent DDT solution or 1 per cent sodium arsenite solution. Straddle trenches, pit latrines and other sources of fly breeding were treated similarly. Mosquito control measures were carried out around headquarters, medical facilities, supply dumps and along communication lines. Details of this work are given in Appendix I.

F. THE TRAINING AND EDUCATION PROGRAM

The training and education program was planned to reach every officer and man in the area on a level consistent with his responsibility. This program fell into two parts, one the work of the area headquarters staff who developed the necessary directives, provided manuals, posters and other educational aids and conducted a small area training center; and the other, the work of base and division malaria control groups who directed the mass education program.

1. The work of the area staff was headed by the Area Training and Education Officer. The area staff prepared six pocket size manuals, three on malaria and one each on dengue, filariasis and rodent control. The three manuals on malaria were written respectively for medical officers, for line officers and for enlisted personnel. About 500,000 copies were printed locally to provide one for every officer and man in the area. The area staff included an artist who produced over a two year period, 15 posters, a monthly pin-up calendar and a weekly cartoon for the Sunday edition of the local mineographed paper. "Malaria Moe" and the Frank Mack versions of a pin-up girl and of an anopheles mosquito were found in nearly every tent and quonset hut in the area. Posters were reproduced in numbers to supply one large and one small size for every two hundred men. One calendar was printed monthly for every five men, Fig. 3.

A library of malaria control films was obtained and circulated to all base and division groups who arranged for command showing of the more important ones. Only two films were considered adequate, a "Snafu" film on malaria and a film produced by the Army Air Corps, TF1-3343. Other films were out-dated, were too technical or were not accurate. Adequate films were not to be had on dengue control, on filariasis or on rodent control. Yet such excellent results were obtained from the few films that were available that it is to be hoped that more and better training films will be prepared for these subjects.

A monthly news letter was found to be a most successful method of disseminating current information to malaria control officers, hospitals, base and division surgeons.

An Area Training Center in Malariology and Other Insect-borne Diseases was established first at Efate and later at Espiritu Santos, depending on the current location of Area Malaria and Insect Control Headquarters. It began late in 1942 with small classes of 3 to 5 officers for 2 week periods. The students at this school included all newly arrived malariologists and such troop unit malaria

control officers as could be spared from their organizations. The school was located on the grounds of a large hospital which had a high census of patients ill with malaria and other tropical diseases and who were available for clinical and parasitological study. The medical staff of the hospital aided the area malaria control staff in the teaching program. Adequate parasitological and entomological collections were built up and a small but good library was obtained.

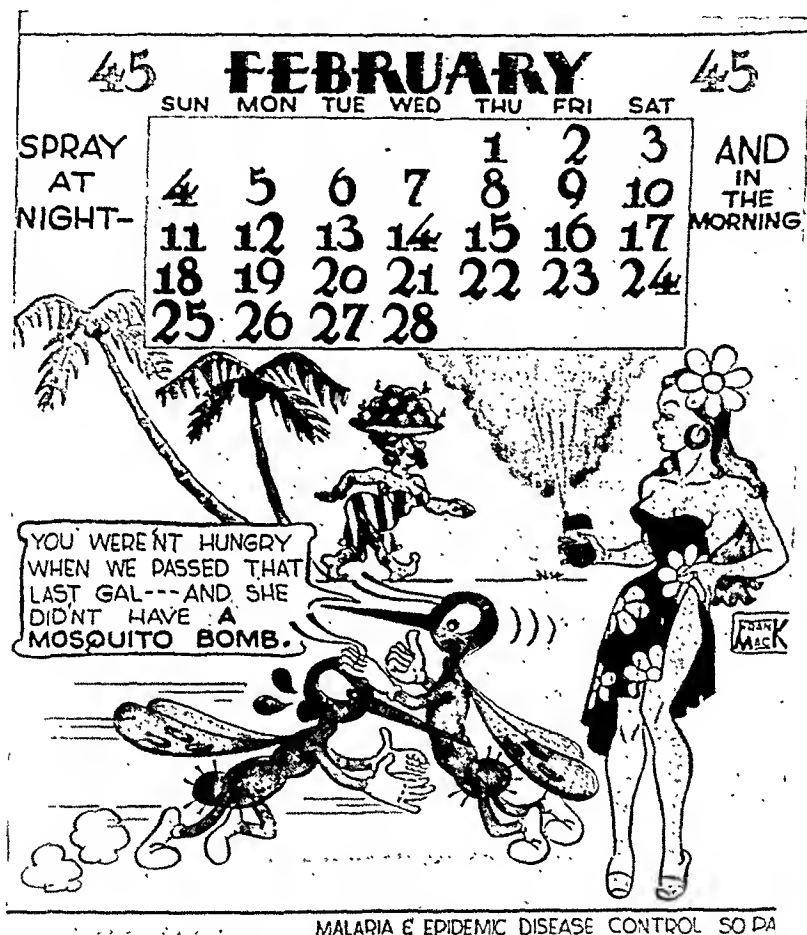


FIG. 3. PIN UP CALENDARS IN FOUR COLORS WERE DISTRIBUTED AT THE RATE OF ONE PER TEN MEN

Thirty malariologists, or more than two-thirds of those who worked in the area, attended this school as did a larger number of troop unit malaria control officers. A small number of entomologists and parasitologists were present at these courses, their work being arranged to give them more time in their specialty and less clinical work.

The daily schedule included ward rounds and clinical conferences, parasitology and entomology laboratory, a lecture and 2 to 4 hours of field work. The curriculum emphasized current problems in the area and included a study of the malaria

rates of each base, division and other adequately observed group. The early attempts to demalarialize large bodies of troops and the later policy to continue atabrine in heavily seeded units during their rehabilitation was studied. Practical experience was obtained with the work of the survey and control detachments and with their records and report forms. Administration and supply problems were discussed with the Area Administrative Officer. The history of atabrine, particularly as a suppressive drug, and the implications of current work on atabrine serum levels were considered. The urgent need for better methods to prevent the spread of malaria to non-malarious bases was stressed. Students took an active part in the local work of the training and education program.

2. The training program on each island or base was divided in three parts, an apprenticeship system for newly arriving personnel of malaria control and survey detachments, a short school for troop unit antimalaria details and a simple educational program for every man in the area.

a. The apprenticeship system was devised to meet the needs of incoming malariologists, entomologists, parasitologists, engineers and the men of their detachments, few of whom were experienced in the problems peculiar to the South Pacific area. This apprenticeship period lasted 1 to 3 months. The teaching staff consisted of the entire personnel of a veteran group (Malariologist, malaria survey and control detachments and labor units) and the curriculum was the daily work of this established group plus a planned training program.

The apprenticed or staging malariologist observed and shared in all the problems of the established malariologist. In addition he worked with the entomologist, parasitologist, and engineer, and their enlisted men in the laboratory and field. When possible, the staging malariologist was given a few weeks of work as head of an established group before being sent to an independent assignment.

The established entomologist outlined to the staging entomologist the methods of work, the system of records, and the peculiar problems of his territory. As soon as possible, the staging entomologist and his crew were assigned a portion of the base and made responsible for all phases of survey work therein. This plan enabled the entomologist to evaluate the abilities of his crew to do field work and to initiate additional training as needed. It was emphasized that this was only an elementary training and that further knowledge and proficiency developed with work. Field men were taught the application of survey to control and that they were the eyes of the larvicidal crews. The need for direct and immediate transfer of survey information to the control crews was stressed. It was emphasized that the field men were often first to recognize the need for drainage or other correction of man-made mosquito breeding sites. They were encouraged to develop the ability to recognize proper corrective measures applicable to such control problems and to make suitable recommendations. Selected individuals were instructed in insectary procedure and mosquito identification.

The engineers and the men of the control detachments went thru a similar apprenticeship. Oftentimes it was possible to place 6 or 8 men of a control unit with a Navy construction battalion or Army Engineer Company, where they

rotated through a program of work with dragline crew, transit crew, dynamite gangs, and heavy maintenance section.

The training of enlisted personnel as technicians qualified to read blood smears for malaria was one of the urgent problems in this early period. The apprenticeship training in this subject served not only the parasitology section of new survey detachments but also technicians from hospitals and other units who needed laboratory workers. The first school for technicians was started at Efate, using as instructors corpsmen who had been trained at the Navy Medical School. Fifty to one-hundred routine thick blood smears were examined each day by these corpsmen and were available for teaching purposes. Technicians of staging hospitals, and dispensaries were trained first, and later technicians of regimental aid stations and battalion sick bays. Similar schools were established on Santo, Guadalcanal, and other bases as soon as malaria control groups arrived. Students were trained either singly or in small groups. The course lasted from 2 weeks to a month, depending upon the background of the individual and the speed with which he became proficient in the work. Over 450 technicians were trained in the first 2 years of this work with an improvement in malaria diagnosis throughout the area to the point where over 95% of all cases were confirmed by the laboratory.

b. The School for Troop Unit Anti-Malaria Details was designed to teach the elements of larviciding and other control measures to the men who comprised these details in each company. This activity was initiated by a directive issued in September 1943²⁶ and revised October 1944 by ComSoPac 02158 which is quoted in part:—

“7. Each unit will arrange with the permanent Base Malaria Control Unit to hold a school for those officers and non-commissioned officers who are designated for malaria and mosquito control work. In planning these schools precedence will be given those units anticipating movement to forward areas. The following subjects will be taught:

- a. Identification of anopheline larvae and adult mosquitoes.
- b. Use of maps to mark breeding places.
- c. Control of mosquito breeding by draining, filling, spraying with oil and use of drip oilers.
- d. Assembly and repair of knapsack oil sprayers.”

An effort was made not only to show how to control malaria, but also to explain the rationale of this work, thus creating a nucleus of informed officers and men in each battalion and company. An average class consisted of 10 to 15 students. The officers and selected enlisted men from base and division malaria control groups comprised the faculty.

The presentation of subject matter was elementary. The unit medical officer was required to attend because he was expected to use this type of presentation in his talks with the men of his organization. Emphasis was placed on practical field work. Between 4000 and 5000 officers and men attended these schools during the first 2 years of their activity, Table X.

- c. Educational program for all personnel. All of the above described pro-

²⁶ ComSoPac Serial 01619, dated 13 September 1943.

grams were concerned with personnel engaged in full or part time insect and rodent control work. The basic educational program, which is now to be described, aimed to impress every man with the importance of malaria and how he might protect himself from mosquito borne disease. Few troops had had any education in malaria before arrival in the area. The early need for this educational work was so apparent that programs were initiated almost simultaneously on several staging bases including Fiji, New Zealand and New Caledonia. The value of these early uncoordinated efforts was immediately evident. At the same time there was apparent need for a uniform area training and education program, for approved training manuals and for a publicity program employing

TABLE X
Attendance at malaria control schools

BASE	SCHOOL ATTENDANCE		LENGTH OF COURSE
	Officers	EM	
			<i>days</i>
New Zealand	100	75	5
Fiji	1	80	7-14
New Caledonia	300	700	1- 5
Efate	20	150	1- 2
Santo	106	574	2- 5
Guadalcanal*	225	1836	3
Russells	42	246	2
Munda-Segi	50	269	1
Bougainville	2	25	3
Green Island	0	25	3
Emiru Island	0	60	2½

* A school was held at each of the 4 sub-groups on Guadalcanl.

the radio and other educational aides. Excerpts from the training directive, ComSoPac Serial 02158, October 1944, are quoted as follows:

“TRAINING PROGRAM IN MALARIA CONTROL

“1. Unit commanders will allot in the training schedules sufficient time for the proper instruction of their troops in the principles of malaria prevention.

“2. Lectures will be given to small groups of men by their respective medical officers. These lectures will cover the following:

- a. Military importance of Malaria.
- b. Nature of Malaria, How Transmitted and Effects.
- c. Individual Protective Measures, Conditions in Which Each is Applicable, Especially in Combat.
 - Repellents
 - Use of Ordinary Clothes for Protection.
 - Spray-Killing of Adult Mosquitoes.
 - Bed Nets
 - Atabrine Suppressive Therapy.
 - Avoidance of Unnecessary Exposure.
- d. Control of Mosquito Breeding.
- e. Man-made Malaria—How to Avoid it.

"3. All personnel will be given initial instruction in prevention of malaria by lectures and motion pictures as soon as practicable. Subsequently, a review of the subject will be carried out at least once a month.

"4. Additional instruction will be given to officers and non-commissioned officers, or petty officers, covering especially the selection of campsites, the hazard of natives as a source of malaria, and the enforcement of precautions under varying field conditions. Emphasis will be put on the responsibility of officers and non-commissioned officers for good "Malaria Discipline," and its importance to military success. Arrangements may be made to have members of Base Malaria Control Units assist in this program.

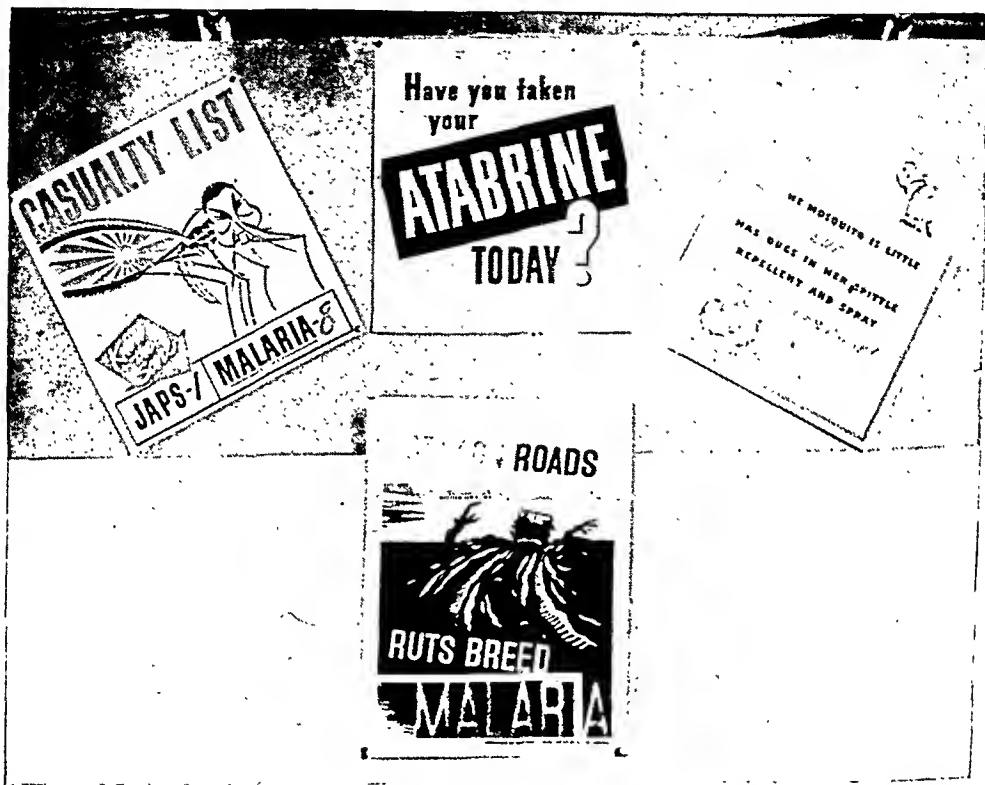


FIG. 4. POSTERS DESIGNED BY ARTIST AT AREA HEADQUARTERS WERE PRINTED AT RATE OF ONE PER ONE HUNDRED MEN

"5. To aid in this program permanently based Malaria Control Units (or Island Surgeons) will issue the following malaria training manuals:

All Medical Officers: MTM No. 1. Prevention of Malaria in Military and Naval Forces, SPA.

All Officers: MTM No. 2. Military Malaria Control, in the Field.

Enlisted Men: MTM No. 3. Malaria, Mosquitoes, and Men.

Movies, additional literature, posters, and other material will also be made available by Malaria Control Units.

"6. Every unit will periodically conduct field exercises in the practical application of antimalarial measures. On maneuvers, these measures will be standard procedures."

Every available educational aid was utilized (figs. 3 and 4). Arrangements were made for brief radio announcements on each base every evening which reminded listeners to roll down sleeves, to use repellent and to take other pre-

cautions. The radio stations were known as the Mosquito Network and on Guadalcanal a program of recorded music known as the Atabrine Cocktail Hour began each evening with a plug for malaria or dengue control.

G. CONTROL MEASURES

The primary preventive medicine problem in the South Pacific Area was the control of malaria; problems of secondary importance were the prevention of dengue fever, filariasis and tsutsugamushi disease. Enormous fly populations developed on all bases where combat was severe and where adequate sanitation was difficult or impossible to attain, yet fly control was not made the concern of the malaria control organization until late in the campaign. The lessons in fly control which were learned here were subsequently of great value in other campaigns where fly control was a major effort.

Before considering the measures employed against larval and adult mosquitoes a brief account will be given of the problems of segregation of natives and of methods to enforce the use of individual protective measures against mosquitoes.

1. Enforcement of individual protective measures

The education of all personnel in the value of individual protective measures has been discussed above and was followed by the publication on each island base of directives and report forms pertaining to malaria discipline, Exhibits I and II.

Each unit commander or his representative was responsible for routine inspections to assure malaria discipline in his unit. Base and division malaria control officers made spot inspections to determine compliance with directives.

EXHIBIT I

HEADQUARTERS FORWARD AREA

APO #.

24 November 1943

Memorandum }
Number 14 }

INDIVIDUAL MEASURES TO PREVENT MALARIA

1. This directive supplements AG 300.6 (Y-P), this Headquarters, dated 27 October 1943.
2. Between the hours of 1800 and 0630, personnel not under a mosquito bar or in screened quarters will wear long trousers and shirts with sleeves rolled down.
3. Use of repellents: Ten to fifteen drops of repellent should be rubbed on hands, wrists, face, and neck at dusk, and will be repeated every three hours when exposed at night.
4. Use of insecticide, aerosol, 1-lb dispensers: All tents should be sprayed before bedtime. Ten seconds spraying is adequate for a pyramidal tent; more is wasteful.
5. Night raids and alerts: Foxholes will be sprayed immediately after entering. Repellent will be taken to foxhole and used as directed in par. 3.
6. All personnel not housed in adequately screened quarters will sleep under mosquito bars properly attached.
7. Swimming, and taking showers in unscreened bath houses, and other unnecessary exposure between the hours 1800 and 0630 are absolutely prohibited.
8. Good Malaria Discipline means that the individual protective measures listed above are continually and consciously carried out by each member of an organization. Such discipline is a command function. Unit commanders will hold their unit malaria control officers responsible for making necessary inspections and reports. Inspectors from Base Malaria Control Units will make spot checks, and report directly to this Headquarters.

By command of MAJOR GENERAL

EXHIBIT II

HEADQUARTERS X ISLAND COMMAND
APO #..........
(Date)

Subject: Violation of Malaria Discipline

To: Commanding Officer,

Ref.: A. Memorandum #14, Hqs, For'd Area, APO, 24 November 1943

1. The violation of malaria discipline by a member of your command or occurring in your camp area, is reported:

- a. ☐ Improper uses of clothing, (no shirt, short trousers, sleeves rolled up, shirt unbuttoned).
- b. ☐ No mosquito bar, or improper use of same.
- c. ☐ Swimming, or taking showers in unscreened quarters after dark.
- d. ☐ Atabrine administration:
 - (1) Inadequate supervision.
 - (2) Failure to maintain adequate roster.

2. Time and Place of Violation:

3. Names of Individual Violators	Organization	Violation (a, b, c, d)
.....
.....
.....
.....

4. Remarks:

5. Officer with whom inspection was made:

6. Malaria Control Inspector:

7. Report of action taken (is) (is not) desired.

By command of MAJOR GENERAL

The usual procedure of the inspecting officer was to walk through the camp after dark, take the names of any men improperly dressed, inspect a few bed-nets, and inquire about supplies of repellent and the atabrine roster.

The malariologist and his assistants were responsible also for inspection of planes and ships to assure compliance with directives designed to prevent dissemination of mosquitoes from one base to another. Weekly spot inspections of outgoing planes were made at all airfields and seaplane bases. Violations were reported to the Island Commander. It should be remarked that inspections made in 1942 and 1943 indicated frequent failure to disinfect both ships and planes. Yet, no anopheline mosquitoes had been found in New Caledonia, Fiji, Samoa, New Zealand or other non-malarious bases as late as July 1945. The malariologist at non-malarious bases was responsible for anopheline surveys around airports and harbors and for investigating cases of malaria claimed to have been contracted on non-malarious bases. Numerous rumors of such cases were all groundless as of July 1945.

2. Segregation of Natives

Surveys presented in Paper IV show that the natives were a serious hazard as a seed bed of both malaria and filariasis. The malaria epidemic index (splenic

index combined with parasitic index) was more than 75 per cent. The micro-filaria parasitic index was more than 20 per cent. The size of the problem is shown in Table XI.

Table XI shows that the indigenous natives seldom were a serious problem on bases occupied by assault, most villages being evacuated voluntarily during the combat period. Movement of villages which were included within the perimeter during peaceful occupation or expansion of a base was more difficult. An official recommendation for removal was initiated by the malariologist and was sent to the Island Commander who made representations to the Colonial authorities. This process was rarely effective in the New Hebrides where the interests of

TABLE XI

*Native seed bed of malaria and filariasis—approximate numbers within one mile of troops**

BASE	MELANESIANS		TONGANESE	SCHOOLS	TOTAL
	Indigenous	Imported Labor			
Efate.....	700	300	200	180	1380†
Espiritu Santo.....	0	300	700	30	1030
Guadalcanal.....	50	2200	0	0	2250
Tulagi-Florida.....	‡	246	0	0	246
Russell Islands.....	400	675	0	0	1075
Segi.....	0	0	0	0	0
Munda & Ondonga Area.....	0	0	0	0	0
Vella Lavella.....	0	0	0	0	0
Treasury.....	180	0	0	0	180
Bougainville.....	0	1600	0	0	1600
Green Island.....	120	0	0	0	120
Emiru.....	225	40	0	0	265
Total.....	1675	5361	900	210	8146

* As of May 1944.

† About 1000 heavily-seeded white Europeans were also within one mile of troops.

‡ 4 villages, population not known, were within one mile of troops.

plantation owners were involved, yet the same method was usually successful in the Solomons where plantations had been abandoned as the Japanese invaded. The natives listed as imported labor were about 70 per cent of the total number of natives within perimeter and were usually brought in to the occupied area from an adjacent island by the colonial labor corps. They were quartered in labor camps. These laborers were urgently needed in the conduct of military operations and the command decision was to use them in spite of their hazard as a seed bed. The chief exception to this policy was on Espiritu Santo where the malariologist persuaded the Island Command not to use native labor. The absence of an epidemic of malaria on this base at the time of the great outbreaks on Efate and on Guadalcanal resulted, at least in part, from the decrease in the seed bed thus accomplished.

The legal basis for dealing with the native problem was found in ComSoPac Serial 5936, dated 29 October 1943, which is quoted in part:

"Island commanders will maintain close watch on potential native sources of malaria and filaria infections. They will make appropriate recommendations to Commander, South Pacific Area, in each case where natives are considered a potential menace.

"Medical Officers of the United States forces are hereby authorized to undertake the treatment of indigenous natives to reduce the health hazard to our forces. The expenditure of medical supplies for this purpose is authorized.

"The above command also applies to natives, imported or employed by civil firms so located as to be a health hazard to our forces."

In the early days of small perimeters and acute shortage of transportation, labor corps camps were located near their place of work, usually a ration or ammunition dump. These locations were often immediately adjacent to troop bivouac sites.

Removal of native labor camps to at least a mile from troop bivouac sites was the goal on all islands; until this could be accomplished reliance was put upon a larvicidal oiling program and drug therapy. Spraying of native huts with freon aerosol pyrethrum "bombs" was initiated in October 1943 when these became available in quantity.

Labor corps officials who assisted in the administration of anti-malarial drugs to natives warned that forcible measures would probably be required to make natives take such medication. However, the writers have personally administered atabrine to thousands of natives without a single refusal by the simple expedient of first publicly swallowing a tablet themselves. The suppressive dose was atabrine 0.1 gm daily. Occasionally this was supplemented by mass therapy and then the dose was atabrine 0.3 gm daily for 7 days. This was followed in the early days by plasmochin 0.020 gm daily for 5 days. Data in Table XII shows that while such therapy reduced the incidence of parasitemia, that parasites were still found in about 2 per cent of natives. The incidence of sexual forms was too low to be significant. Subsequent information on the "curative" action of atabrine on falciparum infections suggests that this therapy was more valuable than was appreciated at the time.

The relocation of native labor corps camps created a continual conflict of interests. The malariologist wanted to remove all such camps a mile or more from troops. The Service Command wanted natives to be located near the site of their work so as to conserve transportation. The colonial labor officers objected to being moved from desirable sites in a populated area to remote and new locations. Despite these difficulties, most of the labor corps natives were relocated by the middle of 1944. This was often an expensive and time consuming procedure, as was witnessed by the removal of a large labor camp on Guadalcanal which required the building of more than a mile of all weather road with drainage system.

The native Melanesians were enthusiastic traders and found our troops to be eager and gullible customers for all kinds of native handiwork. It was suspected that not a few "war clubs" were roughed out on power lathes, polished and

properly marked by native entrepreneurs and sold to the mutual benefit of all producers. However, the shoe was sometimes on the other foot and an occasional pleased and credulous native was seen walking home with G. I. equipment which included an electric light bulb and socket with a foot of attached wire. One suspected that international relations were not improved when he hung up his bulb and turned the button. This urge to trade and to sightsee led to continual violation of regulations prohibiting natives from camp areas after dark and placing native villages "out of bounds" for troops.

TABLE XII
*Effect of therapy on malaria incidence in native laborers**

ISLAND OF ORIGIN	BEFORE TREATMENT		TREATMENT	AFTER TREATMENT†	
	Exam-ined	Positive		Exam-ined	Positive
	No.	%		No.	%
Guadalcanal	106	10	Mass atabrine-plasmochin	204	1
Malaita	241	12	Suppressive atabrine	1022	2
Malaita			Mass atabrine-plasmochin after suppressive atabrine	90	2
San Cristobal	219	7	Suppressive atabrine	400	2

* These studies were carried out on adult males quartered where malaria transmission was at a minimum.

† Examinations made 1 to 10 days after treatment.

Selection of camp sites was closely allied with the segregation of natives and aimed also to locate military installations away from mosquito breeding places. The following paragraphs are from pertinent directives:²⁷

"It is directed that officers in charge of malaria control units be consulted in connection with selection of sites of camps and airfields, and that their recommendations in such matters be given due consideration.

"Information concerning any contemplated troop movement of any force coming to or leaving a malarious base will be made known by the island or force commander to the senior malaria control officer at each base concerned as early as such information is received."

The duties of the malariologist in the selection of campsites included liaison with G-3 concerning contemplated troop movements, survey of proposed campsites with the entomologist and engineer, preparation of a list of suitable campsites of various sizes, preparation of a list of unsuitable regions difficult or impossible to control, with a recommendation that they be declared out of bounds for bivouac purposes and initiation of control work in and around new campsites at least a month prior to occupancy.

The prerequisites of a campsite changed with the tactical situation. Protective cover in the form of palm grove or jungle was a primary consideration under conditions of combat or bombing regardless of increased exposure to malaria.

²⁷ ComSoPac Serial 0094b, 13 November and Hq., USAFISPA, 29 November 1942, and AG 720, Hq., USAFISPA, 24 May 1943.

Open sites, even though less of a malaria hazard were not acceptable until the tactical situation no longer required protective cover. If the malariologist acquiesced in an initial poor location, he was expected to recommend a more favorable location as the tactical situation improved. If a mile between troops and natives was not immediately feasible, a lesser distance was accepted temporarily, and increased as soon as possible. Any distance over a half-mile was of distinct value in decreasing transmission of malaria and filariasis; and even shorter distances decreased the hazard of dengue. The policy of avoiding heavy anopheline breeding sites was recommended whenever compatible with military plans. However, because of restricted beachhead perimeters and the military importance of rivers it was rarely possible to locate troops a mile or more from swamps, river deltas, and other anopheline breeding areas. Rather, it was necessary to control mosquito breeding in such regions.

It was also the duty of the malariologist to suggest relatively malaria-free regions for night maneuvers and amphibious landing drills. He surveyed the regions which were available for such tactical operations with the entomologist and informed G-3 of those which were suitable. He advised that highly malarious territory be placed out of bounds for night exercises until it could be brought under control. Even when night exercises were held in authorized sectors, all anti-mosquito precautions were regularly enforced, both for protection and as a training measure.

3. Larvicidal Work—Ground Application

It is worthy of note that larvicidal operations were well developed on all bases before DDT became available. Larviciding was usually the first type of insect control to be done on a new base. Detailed maps were essential for this work and if available, aerial mosaics were studied before arriving. These maps were corrected and new information added each day by both survey and control personnel. The initial larviciding rapidly progressed to a routine oiling program which usually followed a weekly schedule. Insect control work in territory occupied by troop units was assigned to the anti-malaria details of those units. The remaining territory was controlled by labor directly available to the malaria control engineer, Table IX. The engineer divided the labor available for larviciding into crews of 5 to 15 men and assigned to each crew a section of territory which it was to larvicide completely in 4 to 5 days under optimum conditions. This allowed a safety factor for breakdown of equipment, bad weather and other problems, while still permitting a weekly schedule. Men were given 1 day each week for recreation; although in urgent situations, especially in the wet season, a 7 day work week was customary.

"Shock" oiling crews were organized on some large bases to control urgent breeding situations without interfering with the routine of regular crews. Oil depots were established at locations selected by the engineer and his foreman to facilitate oiling and to decrease transportation.

The clearing of stream banks (figs. 5 and 6) and of stream beds was an essential preliminary to adequate oil coverage. This work started with the initial larvi-



FIG. 5. STREAM CLOGGED BY DEBRIS AND COVERED WITH ALGAE MAT BEFORE CLEARING



FIG. 6. SAME SITE AS IN FIGURE 5, AFTER CLEARING. SHADE FOLIAGE HAS BEEN PRESERVED

ciding and was carried on simultaneously. Since sunlight encouraged the breeding of *A. farauti*, it was important to preserve as much shade as possible. Trees were not removed and only a 3 or 4 foot margin was cleared along stream banks to make a path for oiling crews. Natives, working with machetes, were superior to any other labor for this work. Bulldozers were less desirable since they needed a large area in which to operate and cleared a strip 20 to 50 feet wide. Revegetation of cleared areas made reclearing necessary at intervals of 3 to 5 months. This maintenance work was usually done in a fraction of the time required for the original clearing.

Light diesel oil, (Quartermaster item, 7-0-200, oil fuel, grade FS2) was the chief larvicide used in this theater until the introduction of DDT. Table XIII

TABLE XIII

Monthly consumption of diesel oil for larviciding—comparison of dry and wet season reports

BASE	AREA TO BE CONTROLLED	DRY SEASON*	WET SEASON†
	<i>sq. miles</i>	<i>55 gallon drums</i>	<i>55 gallon drums</i>
Efate.....	39	180	250
Espiritu Santo.....	40	255	360
Guadalcanal.....	110	650	1800
Tulagi-Florida.....	9	70	120
Russells.....	15	90	270
New Georgia.....	20	50	100
Vella Lavella.....	20	30	100
Bougainville.....	30		85
Green Island.....	15		90
Emiru.....	30		100
Totals.....	328	1325	3275

* From reports for October and November 1943.

† From reports for January and February 1944, except for Green and Emiru.

Note: When the amount of oil used by troop units was not reported the island figure was increased by approximately 20 per cent.

shows the comparison between amounts used in dry and wet seasons. No DDT was used in the periods reported in Table XIII.

Paris green eventually was completely replaced by DDT. Paris green was used chiefly as a temporary measure along grassy stream margins and the edges of swamps until proper clearing could be instituted in such places. Relatively small amounts were used. Lack of a suitable diluent and of good dusters were the chief obstacles to the wider utilization of paris green. Condemned flour was the most commonly used diluent and became lumpy and unsatisfactory because of the high humidity and contamination by mold and bacteria. No lime was available.

Kerosene and unleaded gasoline were used to treat native wells and water tanks which could not be closed or screened. When applied in the evening the kerosene evaporated before morning and did not affect the potability of the

water. The amount applied was 4 ounces of kerosene per 100 square feet of water surface.

DDT began to arrive in quantity about the middle of 1944. DDT in oil was used as a 5, 2.5 and 1 per cent solution. Since each type of spray equipment delivered a different minimum output of DDT-oil solution the concentration was varied accordingly. The minimum concentrations of DDT in oil shown in Table XIV gave nearly 100 per cent kill when adequately applied.

The use of DDT resulted in great economy of labor and of diesel oil. A medical sanitary company on Guadalcanal equipped with flit guns, reported that their monthly output of DDT-oil solution to larvicide a stipulated area was 150 gallons. This amount represented 10 per cent of the 1500 gallons of plain diesel oil formerly used to cover the same area with knapsack sprayers. Others advantages of DDT-oil over plain diesel oil were the decreased weight carried by

TABLE XIV
Relationship of DDT concentration to minimum output of equipment
Guadalcanal, June-October 1944

EQUIPMENT	MINIMUM OUTPUT	RECOMMENDED MINIMUM CONCENTRATION	
		Per cent	Lbs DDT per acre
	<i>gal./acre</i>		
Knapsack and C. W. Decontamination Sprayer.....	5-7	1	0.48*
"Flit Gun" type sprayer.....	1.2-2	2.5	0.3*
Airplane.....	0.5	5	0.2

* Pounds of DDT/acre for "Flit Gun" sprayers calculated on output of 1.5 gal/acre; for knapsack and C. W. decontamination sprayers on output of 6 gal/acre.

each field man and the less frequent filling of sprayers and replenishing of oil depots.

The introduction of DDT did not alter the weekly larvicidal schedule, although heavier applications of DDT in a few static pools did give a residual larvicidal effect for longer periods. The chief breeding areas, however, were of a nature which did not lend themselves to larviciding for residual effect. This fact and the heavy rainfall during the wet season made it necessary to maintain the weekly larvicidal schedule for the major portion of control work.

DDT dusting preparations were used much less than DDT-oil solutions. Ten per cent DDT dust gave excellent larvicidal action when dusted on small water surfaces such as road ruts or rain barrels and survey men usually carried a 2 ounce can to treat small breeding places when found. Dispersion of DDT dust over large water surfaces was frequently handicapped by unsatisfactory dusting equipment. In addition to the same difficulties with diluents for DDT, as is noted above for paris green, it was reported that DDT and lime were chemically incompatible. The 10 per cent DDT dusting powder was ordinarily used without dilution.

The equipment for application of DDT-oil solutions will be discussed briefly. Knapsack sprayers were available in 3 and 5 gallon sizes. The Chemical Warfare Item, Apparatus, decontamination, 3-gal cap., M-1, pressure type sprayer when modified with a suitable nozzle and oil-resistant hose was the lightest, most durable and generally satisfactory knapsack sprayer. It was reasonable to standardize on this one sprayer for both insect control and chemical warfare uses. The natural rubber hose on both of these sprayers deteriorated rapidly after contact with oil and small particles of rubber broke off and clogged the screen, whirl plates and spray disc. Neoprene or other oil resistant hose solved this difficulty.

The "Flit Gun" type sprayer²⁸ which was equipped with an atomizing nozzle was one of the most useful instruments available for ground application of DDT-oil solutions. This sprayer delivered a fine spray which was effectively applied in still air or when aided by a wind drift. Operators were trained to take advantage of wind direction to obtain maximum coverage with minimum amount of larvicide and effort. A visible film of oil was not always detectable on the water. The best criterion of coverage was a larval survey before spraying and 24 hours after spraying. Surveys made less than 24 hours after spraying often gave erroneous results because of the delayed killing action of DDT.

Aerosol generators²⁹ were tried extensively on Guadalcanal and proved to be of value both for larviciding and for killing of adult mosquitoes but their use was limited by the need for particular meteorological conditions and by mechanical problems of operation.³⁰ This generator was also useful for sand-fly control along an occupied beach.

Mixing of DDT with oil became a problem as its use increased. Simple hand mixing gave way to various types of mechanical agitation; one of the best being improvised from an orchard sprayer. Larger scale mixing plants made use of compressed air which was released at the bottom of a steel cube or other suitable container and so agitated the oil-DDT mixture.

4. Larvicidal Work—Airplane Application

Airplanes were first used to disperse DDT solutions in the Spring of 1944.³¹ The work was carefully controlled by surveys of larval and adult mosquito populations and observations on droplet size and dispersion.³²

Both small and large airplanes³³ were used in this program and each was fitted with a suitable spray apparatus. Small planes were both valuable and eco-

²⁸ Listed as QM item 41-S-40105. Sprayer, liquid, insect, continuous spray, 2 qt. size.

²⁹ Hochberg La Mer Insecticidal Generator.

³⁰ Newsletter #20 and 22, Hq., Malaria and Epidemic Control, South Pacific Area, February 1945. Abstracts from report of Bohart et al.

³¹ Mr. C. N. Husman and officers from Naval Medical Research Unit No. 2 aided in the initiation of this work.

³² Fallander, S. R.: Analysis of Aircraft Spraying and Equipment in Malaria Control. A.G. 452 (Y-P) Headquarters Guadalcanal Island Command, 23 March 1945.

³³ Maple, John D., The Spraying of DDT from Aircraft, Newsletter 17, Hq., Malaria & Epidemic Control, SoPac Area, November 1944.

nomical as an adjunct to ground coverage. The maneuverability of small planes was an asset in covering small areas which were difficult of access to ground crews. Cub type planes were used to larvicide a field of uncharted land mines, a series of dense coastal swamps and jungle tracts where mosquito breeding was increased by logging operations. Larger, faster planes were valuable to obtain partial and temporary mosquito control over large areas of newly occupied terrain during the period when ground crews were establishing control.

a. Small planes.—The equipment for use in small planes was known as the Husman-Longcoy spray apparatus and was designed at the Bureau of Entomology, U.S.D.A., Orlando, Florida, for installation in the 65 h.p. Piper Cub airplane.³⁴ An improved light weight spray apparatus was later designed and used on Guadalcanal and Espiritu Santo (fig. 7). The capacity of the spray tank was 25 gallons which was adequate to spray 50 acres. The area which a cub plane could cover was adversely affected by rain, which decreased visibility, and by winds in excess of 15 miles per hour, both of which made low flying dangerous. It was estimated that average conditions would allow a single cub plane to cover up to 300 acres per day for a 4 day week; under ideal conditions this might be increased to 400 acres per day. The remaining 3 days allowed for inclement weather, plane servicing, and for miscellaneous delaying factors. Flight lines were 40 feet apart regardless of wind or altitude, but it was desirable to fly cross-wind to take advantage of drift. Flag men were used to indicate these flight lines only until the pilot was able to maintain parallel flights at 40 foot intervals. The flight altitude averaged 25 to 35 feet over open grasslands and 125 feet to 150 feet above the ground over palm groves and jungle.

A ground crew of 2 to 3 men with a $\frac{3}{4}$ ton truck and a tank trailer was equipped to service as required each 1 or 2 cub planes. This truck unit carried a motor driven fuel pump, gasoline, DDT-oil solution and other necessary equipment and made it possible to use small temporary landing fields near the site of spray operations. The number of spray flights which a plane could make each day depended largely upon the efficiency of the ground crew in servicing the plane.

b. Large Airplanes.—TBF and TBM planes were the larger planes which were adapted for airplane application of DDT solutions. The 2nd Marine Airwing used a TBF plane for spraying during early operations on Pelelin Island. An improved apparatus was subsequently designed and used on Guadalcanal. All equipment was standard for this airplane or available on the island. DDT solution was carried in the standard 265 gallon belly tank. A speed of 115 knots with 15 degree flaps was selected as giving the best distribution while maintaining maneuverability of the plane. A swath width of 150 feet gave a uniform deposit of 2 qts. per acre. At this rate of 2 qts. per acre the 265 gallons in the belly tank covered approximately 500 acres. The minimum time required to spray this amount was about 13 minutes. Field tests with both the L4B and the TBM repeatedly showed that the larvicidal action of 2 qts. per acre of 5 per cent DDT solution in oil was equivalent in larvicidal effect to careful ground coverage.

³⁴ L4B and AE-1 were the army and navy designations for the two types of planes which were used.

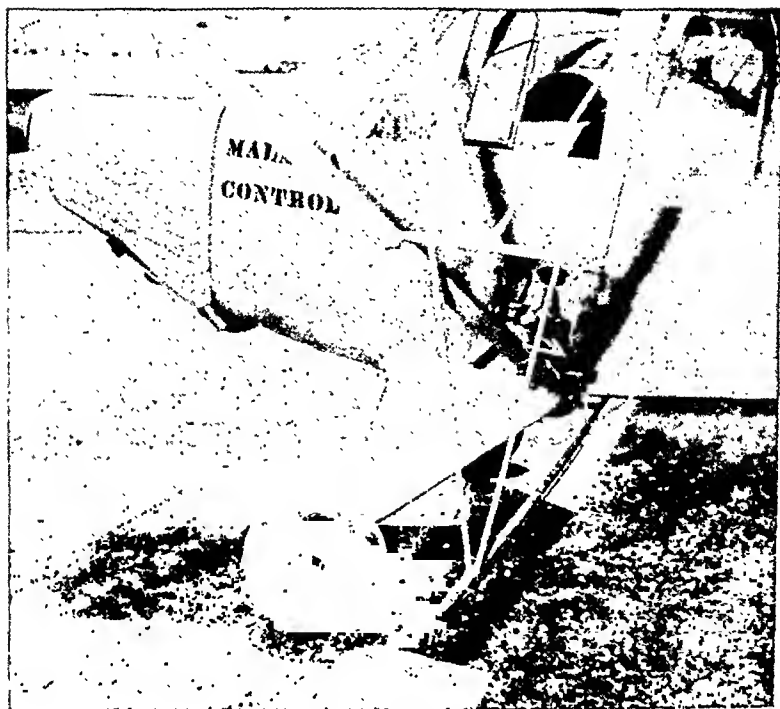


FIG. 7. AE-1 PLANE SPRAYING DDT SOLUTION. NOTE TWELVE FOOT SPRAY BAR UNDER FUSELAGE

5. Measures Directed against Adult Mosquitoes

Spray killing of mosquitoes was almost entirely limited to the use of pyrethrum aerosols, and to insecticidal preparations of DDT. Pyrethrum insecticide, aerosol, 1-lb and 5-lb dispensers were used to kill mosquitoes in bed nets, screened buildings and covered foxholes. The value in open foxholes and unscreened tents was transitory. Aerosols were little used in combat areas. They were used to spray native huts located near troops. Spraying was done each morning, and a native dresser was trained in the work. This method of mosquito control was effective because of the habit of *Anopheles farauti* to rest for several hours in the hut or tent where the blood meal had been recently obtained. The plan of weekly spraying, as applied to *A. culicifacies*, Giles (12), was not thought adequate because there was no evidence of prolonged resting of *A. farauti* in hutments.

DDT preparations designed for residual effect were applied to the inside wood-work and screening of tents and buildings and to the palm leaves and bamboo stems of the interiors of native huts. The most satisfactory application was made by means of a power driven paint sprayer. One pint to one quart was applied to each 250 square feet of surface. A satisfactory rule of thumb was to spray the surface to wetness: i.e., a pattern of closely packed droplets, but not to the extent that the liquid would run from the sprayed surface. Best results were obtained by running the engine at slow speeds and regulating the pressure to less than 50 pounds per square inch. The tendency of untrained personnel was to use higher spray pressures of 150 to 200 pounds per square inch which atomized the spray and gave less satisfactory coverage.

The effect of residual spraying with DDT on living quarters was remarkable. Mosquitoes and flies observed to light on a treated surface developed toxic symptoms within 1 to 2 hours and died within 6 to 24 hours. Ants previously very annoying in tents and huts disappeared. A single application was effective for about 3 months depending on exposure to weather.

Bed nets impregnated with 5 per cent DDT in kerosene. Tests on impregnation of bed nets with DDT were begun in the latter part of 1944, and the methods then developed were used to treat the bed nets and jungle hammocks of 2 divisions. A 5 per cent solution of DDT in kerosene was applied by a power driven paint sprayer or by Chemical Warfare decontamination sprayers, provided with whirl plates and spray disc nozzle to deliver a large size droplet. The bed nets were arranged in piles. The top net was sprayed on one side, reversed to start a new pile and then sprayed on the other side. Six men were able to spray about 60 to 100 nets per hour with hand sprayers and twice this number with a power driven sprayer. This included the preparation of nets, spraying, and the hanging in the sun to dry. One gallon of solution treated 6 to 8 nets.

Treated nets were stored for over a month and then hung over a cot in the usual manner and observed to determine the lethal effect on *A. farauti* which were liberated within the net. Ninety-three of 94 mosquitoes were dead within one hour while no control mosquitoes died in an untreated net. In another test 224 mosquitoes were liberated within the treated net for 10 minutes or less, and were

then recaptured and placed in untreated cages. All were dead within 24 hours of this brief exposure, while no controls died. In the second test an observer remained in the treated net with the mosquitoes and noticed that they attempted to bite during the first 2 minutes, but not after 5 minutes (13).

All the bed nets of one division and the jungle hammocks of another division were treated with DDT solutions during the last weeks prior to departure for uncontrolled, malarious areas. The effect of carrying bed nets in barracks bags where they were subject to frequent handling was not known.

Bed nets proved to be the most valuable single measure against mosquito bites. There were instances on Guadalcanal and other bases during the height of the malaria epidemics where less than a week of bivouacking without bed nets resulted in a high infection rate among unprotected troops. Orders were issued making it the responsibility of each officer and man that bed nets should be available on the first night ashore except among troops actually in combat. Compliance was generally excellent after the early costly experiences. The educational program continually emphasized the importance and the proper use of bed nets. The early shortages of bed nets and particularly of replacements was part of the total supply problem. Replacements of bed nets was adequate on rear bases from the fall of 1943 and on all bases by the end of that year.

Jungle hammocks became available and were issued to many troops including whole divisions beginning late in 1943. These hammocks when properly suspended were excellent and facilitated net protection in forward areas and outposts. However, the issue of jungle hammocks to entire divisions was of doubtful value because of two reasons. First, because of their bulk they were one of the first items to be discarded during combat operations. Secondly, it was rarely possible for any significant number of men in a division to find trees or other supports adequate for the suspension of these hammocks.

Head nets were issued to all troops and were not used. The use of head nets for protection during sleep was not practicable because the movements of the sleeping individual soon disarranged the head net or brought it against his face and so allowed mosquitoes to bite through the meshes. The routine issue of head nets was not warranted under conditions prevailing in the South Pacific.

Repellents. Only 612 and dimethylphthalate, of the available repellents,³⁵ were effective against the *Anopheles farauti*. 612, when adequately applied repelled anophelines for about 2 hours. Dimethylphthalate was effective for much shorter periods. Profuse sweating decreased this repellent effect. Combat troops frequently claimed that Japanese could smell repellent for many yards if the wind was in the right direction. Service troops disliked the messiness of a repellent and used it only when mosquito bites were annoying or when malaria discipline was strictly enforced. Repellents were potentially a valuable individual protective measure but were relatively little used. Supplies were adequate from the middle of 1943 and a surplus supply in the area of over 12 million bottles by the end of 1944 was evidence of lack of use.

³⁵ The new standard Q.M. repellent "6-2-2" was not available until 1945 and was not tested. 6 parts dimethylphthalate, 2 parts Rutgers 612, 2 parts Indalone.

Screen, cloth bobbinette and wire. Screen was slow to arrive. By early 1944 it was available in adequate amounts in all except forward and combat areas. Table XV shows the relationship between length of occupation of each base and the per cent of screened quarters as of June 1944. Cloth bobbinette was preferred to wire for field use and for use on installations near enough to the shore to be subject to wind-blown salt spray. Under these latter conditions cloth bobbinette outlasted wire. It was lighter to ship, required less space per cubic yard, and used no critical materials. Wire or plastic screen was preferable away from the shore in semi-permanent buildings such as hospital wards and mess halls. When screen was limited in amount, the following priorities were established: hospitals; kitchens and mess halls, showers, particularly for organi-

TABLE XV
Estimated per cent of quarters screened as of 1 June 1944

BASE	DATE OF OCCUPATION	HOSPITALS ARMY-NAVY	KITCHENS & MESS HALLS ARMY-NAVY	SHOWERS		TENTS	
				Army	Navy	Army	Navy
Efate.....	Mar. '42	100-100	100- 95	90	90	100	100
Santo.....	May '42	100-100	100-100	90	95	90	90
Guadalcanal.....	Aug. '42	100-100	90- 90	65	70	90	90
Tulagi-Florida.....	Aug. '42	100-100	100-100	100	100	100	90
Russells.....	Mar. '43	100-100	40- 40	5	5	10	10
Munda.....	July '43	100-100	100-100	10	20	40	30
Bougainville.....	Nov. '43	100-100	100-100	50	15	5	5
Green Island.....	Feb. '44	100-100	100-100	40	25	15	10
Emiru.....	Mar. '44	100-100	100- 95	75	25	20	2

Note: Navy data includes that of Marines.

zations with men on night details; latrines; offices, tents, and all other living quarters. The recommended initial allowance was 16 linear feet of screen bar per man and 4 feet per man per month as replacement.

6. *Semi-Permanent and Permanent Control Work*

Filling. Most filling operations were concerned with the elimination of man-made breeding places such as foxholes, borrow pits, bomb craters, and road ruts. Foxholes along the shore and especially in coconut groves were filled and levelled most economically by bulldozers. Foxholes in the jungle usually were hidden along lines roughly parallel to streams. Finding and filling these was a project best done by native labor.

Drainage: Hand ditching. Medical sanitary companies were organized with ditching platoons and on some bases dug many miles of excellent ditches which greatly reduced the work of larviciding. Small feeding ditches in a bivouac site were made with hand labor by the troops concerned. Large main drainage ditches were not usually dug by troop labor. The full potentialities of hand ditching were realized only rarely as in the 37th Division Area on Bougainville. Survey and oiling crews were urged to be alert to the possibility of hand drainage

A charge of dynamite exploded in the bottom of such a silted-up drainage hole often corrected the condition.

A unique type of vertical drainage was used on Green Island to aid in the elimination of extensive road ruts. These ruts averaged 6 inches in depth and contained water which had to be eliminated before the ruts could be filled. Pools of water in the road were drained by forcefully driving an iron rod down through the water and thin layer of soil into the underlying porous coral. A single strong blow often sank the rod to a depth of 6 feet or more, and as much as 20 gallons of water were drained rapidly by multiple holes. The space thus freed of water was filled in by a crew of hand laborers and the road closed to traffic. About 75 miles of ruts were treated in this manner.

Road drainage. A faulty road drainage system or lack of any road drainage was one of the chief causes of man-made malaria in the early months. Not only were borrow pits dug without provision for drainage but roads were built across natural drainage courses without any provision to care for the water thus impounded. In the wet season thousands of acres of breeding area were created as well as more acres of road ruts. The solution to this problem was adequate roadside ditches with culverts placed to grade. Satisfactory drainage required that a ditch be deep and large enough not only to drain water from roads but also to drain adjacent fields and camps. On bases occupied in the latter part of the campaign this problem was largely avoided by proper construction of roads. Fill for these roads was obtained by digging wide gutters on both sides, only down to drainage grade rather than from deep borrow pits. Roadside ditches were put in simultaneously with the roads and culverts placed to grade. On Emirú, an island policy was established that all roadsides should be drained either to the sea or to a natural drainage course and that this was the responsibility of the road construction group.

Road ruts in little travelled secondary roads, in abandoned jungle roads, and in dumps of all kinds were a great source of anopheline breeding. A heavy disc harrow pulled by a tractor (fig. 9) was the most efficient means of filling and smoothing these ruts, except in very wet ground. The discing leveled the ruts, pulverized the soil and encouraged growth of grass and other vegetation. Roads which no longer had military value were blocked with barbed wire and by the use of suitable signs. The use of these signs was approved by the Commanding General and they were erected in cooperation with the Provost Marshal.

Concrete Inverts. Concrete inverts and other types of permanent lining for ditches were not used on any base in the South Pacific because no concrete was available. Many Islands were occupied, developed and abandoned in such a brief period that such permanent work would have been inadvisable even if materials had been at hand.

Flumes. Lagoons and swamps just back of the beach offered the worst breeding problems on Guadalcanal. Similar problems existed on Bougainville, Green Island, Emirú, and other bases. On Guadalcanal there were over 60 such lagoons in the occupied territory. These were formed by wave action which built up a sandbar across the mouth of a stream in such a way as to form a dam.

The water within the lagoons was increased by rain and stream water to raise the level as much as 3 or 4 feet above sea level, thus providing an ideal breeding place for anophelines. Cutting ditches through the sandbar offered only a temporary solution. The first flumes were located at the narrowest point in the sandbar between the lagoon and the sea. This was the weakest point of the sandbar and at flood time the water often broke through to the sea and washed out the flume. Subsequently, flumes were located at a stronger place in the beach, well to one side of the flood point. The lagoon end of the flume



FIG. 9. DISC HARROWS WERE USED TO ELIMINATE RUTS IN DUMPS, IN ABANDONED ROADS AND IN FIELDS. NOTE RUTTED GROUND TO LEFT OF TRACTOR

was set up so that it was approximately 6 inches under water at mean low tide. The flume was extended on a horizontal through the sandbar and into the sea until it was approximately one foot above the floor of the sea. It was held by strong pilings set at 5 foot intervals on each side of the flumes using a water jet from a 500/gal/minute fire pump. An experienced crew of 14 men with dragline, bulldozer, and fire pump could install a 2-drum flume 200 feet long in 4 to 6 days. No high priority materials were used.

Well placed flumes usually lowered the water level 1 to 3 feet and reduced water area by 70 to 80 per cent, particularly when combined with channel dredging above the lagoon end of the flume. The remaining water was more accessible to oiling. Tidal fluctuation and resulting salinification prevented growth of bank vegetation and mosquito breeding for several hundred feet

inland from the flume. Closure by debris and shifting sand made the maintenance of flumes a constant problem.

Flushing Dams. Dams were constructed on suitable streams to build a head of water which, when suddenly released, rushed down the stream bed, washed out larvae and flotsam and retarded growth of bank vegetation. Flood waters in the rainy season often washed out these dams; therefore, the gate was removed during this season. Seven flushing dams were constructed on Guadalcanal and 2 on Efate.

Biological Control. Early in 1943, 2 shipments of gambusia were obtained from New Zealand. Rapid propagation of these fish supplied the requirements of all bases. Gambusia were placed in swamps and pools on Espiritu Santo, Russell Islands, and Munda where their chief value was to retard anopheline breeding in marginal territory just beyond the limits of the controlled area. They did well in those sink holes on the Russell Islands where the amount of impounded water was large enough to remain fairly fresh and did not evaporate in the dry season. They were used in wells and cisterns on Efate. It must be emphasized that Gambusia required constant supervision and continual maintenance of the pools and swamps where they were placed. They played a small but distinctly useful part in the total larvicidal program.

7. *Suppressive Medication*

a. *Experiences with atabrine.* Atabrine was eventually established as the best available drug for the suppression of vivax malaria and as a true causal prophylactic of falciparum malaria and so occupied an important but never predominant place in the control program. The use of atabrine for the suppression of malaria was made necessary by the Japanese conquest of the sources of quinine and proved to be a fortunate occurrence, although this fact was not immediately apparent. Medical officers concerned with the discovery of the truth about atabrine were confronted with a great lack of precise information. Little was known about absorption, blood concentration or excretion of the drug, nor was it known whether or not prolonged use would lead either to transient toxicity or to permanent injury.

Throughout 1942 and 1943 there was confusion, disagreement, and uncertainty regarding the use and dosage of atabrine. Standard treatises upon malaria warned that atabrine was a dangerous drug and that its use should be controlled by rigid observation. Directives ordered the routine use of atabrine as the basic anti-malaria drug in order to conserve the rapidly diminishing reserves of quinine. This fostered the suspicion that atabrine was a drug which was necessary rather than desirable. Furthermore, the administration of atabrine was frequently begun on shipboard as troops approached malarious islands, and seasickness, diarrhea and emotional states were attributed to a drug already in doubtful favor. The appearance of the skin, tinted a sickly yellow hue, though harmless, led to the assumption that atabrine was injurious to the liver and this feeling was intensified when infectious jaundice appeared in epidemic proportions among troops taking atabrine. Added distrust of the drug arose

from the development of malaria, for reasons which will be given subsequently, despite the presumed administration of suppressive atabrine. When clinical malaria was treated with atabrine and promptly relapsed, both medical officers and competent non-medical personnel, accustomed to regard quinine as a magic cure for malaria, wondered if atabrine were an adequate substitute.

When the Marines entered Guadalcanal in August 1942 there was wide divergence of authoritative opinion regarding the value and the dangers of atabrine suppressive therapy. Some units took quinine, others took atabrine, and many admittedly took nothing. There was no organization, such as is known to be necessary to supervise the administration of suppressive therapy. Soldiers knew that an attack of malaria might hasten their evacuation to a comfortable and safe rear base and this added to the difficulties in administering suppressive therapy. The malaria rates on Guadalcanal at this period are given in Table I.

The area directives on the subject of suppressive atabrine illustrate growth in knowledge concerning this drug. The following excerpt is quoted from the first directive on suppressive atabrine to appear in the South Pacific Area, September 1942, and is indicative of the paucity of reliable information concerning atabrine and the suppression of malaria at that early date.

"Malaria Prophylaxis. It is recommended that malaria prophylaxis be given as follows: Atabrine is the drug of choice. It should be given in doses of 0.2 gram twice weekly (0.4 gram per week). When atabrine is used it is to be considered advisable, after 3 months, because of slight cumulative effect of the drug to substitute quinine for a period of 1 month. Quinine is given prophylactically in doses of 15 grains daily. This should be continued for one month and then atabrine . . . resumed."

In October of 1943 a new directive maintained the suppressive dose of atabrine at 0.4 gram per week, but the schedule was changed so that the drug was taken as follows; $\frac{1}{2}$ tablet (.05 gram) per day on each day of the week except Sunday when 1 tablet (0.1 gram) was taken. In January 1944 the weekly suppressive dose was increased to 0.6 gram of atabrine per week; 0.1 gram each day except Sunday. In November 1944 the last area directives³⁶ were issued on this subject and stipulated that 0.7 gram per week be the authorized suppressive dose to be taken as 0.1 gram each day of the week.

The value of atabrine as a suppressive drug was gradually established by clinical observations. It slowly became apparent from the conflicting clinical reports that a few heavily seeded units which had good atabrine discipline actually were suppressing a great share of their malaria so long as they continued atabrine. Thus the 6th Marines reported less than 250 cases of malaria while on atabrine during January and February 1943, on Guadalcanal, as compared with over 2500 cases in May and June, after they had moved to New Zealand and had discontinued the drug. This organization was given loading doses of atabrine prior to arrival on Guadalcanal and 0.6 gram per week while there,

³⁶ Memorandum #183, Hq., SoPacBaCom, dated 1 November 1944 and ComSoPac Serial 02259 dated 10 November 1944:

with excellent supervision and it was felt that they probably had adequate blood atabrine levels during a period when this was uncommon.

Another organization, the 147th Infantry,³⁷ took suppressive atabrine of 0.4 gram with poor to fair supervision and had a malaria rate during 5 months on Guadalcanal which ranged around 1000 per 1000 per annum. This rate promptly rose after discontinuation of atabrine in non-malarious Samoa to an average of over 3000 per 1000 per annum for 5 months with peaks as high as 14,000 per 1000 per annum in selected groups. Atabrine was then resumed with excellent supervision and the rate dropped abruptly to well under 100/1000/annum. The history of this organization has been reviewed recently, (14).

This clinical data was confirmed by the careful observations and blood atabrine studies of Baker, Shaffer & Lewis³⁸ which showed that the development of clinical malaria in troops supposedly taking atabrine suppression was associated almost invariably with extremely low values for serum atabrine concentrations. Further investigation strengthened their opinion that a low atabrine level was almost always due to laxity in taking the prescribed dose of the drug.

This reasoning was confirmed by reports on "Investigations of Atabrine" by the Armored Medical Research Laboratory, Fort Knox, Kentucky, by the work of Shannon (15), by the conclusive clinical and laboratory experiments of Fairley (16), and by other reports (17, 18).

Administration of atabrine for suppression was ordered to be by roster for both officers and men. An officer or a non-commissioned officer was detailed to watch the actual swallowing of the drug by each individual. The roster was checked daily and all individuals who had failed to take the drug were required to report and to take sufficient dosage to equal the amount missed. Men on patrol or other detached duty were given sufficient drug for the period they were to be away and explicit directions for taking it.

As noted above, the dosage which was finally ordered was 0.7 gram weekly, usually given as 0.1 gram daily. An alternative procedure was to give the drug on 2 days a week in doses of 0.4 gram and 0.3 gram. This was done by the 25th Division during the latter part of 1944. This procedure did not give as even a blood level but did facilitate administration and was quite successful.

Many men were quite adept in circumventing these directives usually by palming the drug or by tucking the tablets between teeth and cheek. The only true solution of this disciplinary problem was an educational program to impress every man with the need for and value of the drug and with its harmlessness.

Toxicity: Temporary and minor gastro-intestinal upsets were not uncommon when atabrine was commenced. Information regarding this possibility was publicized in directives and in educational material. Medical officers were advised to continue the drug in lower dosages for individuals so affected. It was found that less than 1 person in 1000 was intolerant of the drug as prescribed. A few cases of skin lesions which simulated lichen planus (19) were noted in

³⁷ See Graph IX, Paper II.

³⁸ Unpublished reports to the Surgeon, USAFISPA.

1944. Severe toxic manifestations including exfoliative dermatitis and hepatitis were rare, particularly in those who took only the prescribed dose of 0.7 gram weekly.

b. *Atabrine suppressive therapy, discontinuation of.* Atabrine suppressive therapy was discontinued in lightly seeded troops throughout the South Pacific Area as malaria control measures became advanced enough to permit doing so without danger of significantly increasing malaria rates. This policy was initiated in September 1942, and is further delineated by Memorandum No. 183, Hq., SoPacBaCom, 1 November 1944 and by ComSoPac Serial 02259, dated 10 November 1944. Part of the latter directive is quoted:

"4. Island Commanders are authorized to discontinue atabrine suppressive treatment in selected "lightly seeded" units, upon recommendation of the Island Malariologist, as control measures become sufficiently advanced to permit doing so without interfering with the military effort.

"5. Suppressive treatment may conceal the actual amount of infection or the gradual seeding of a unit. Apparent freedom from malaria may lead to a false sense of security and carelessness in regard to truly preventive measures, such as mosquito control and individual protective measures. Therefore, the eradication of the anopheles mosquito and protective measures against it must be continued with unabated energy."

The events which led to this policy are presented. On Efate a fall in the malaria rate from 2600/1000/annum in April 1942 to 144/1000/annum in September 1942 led to an island order to discontinue atabrine. The rate in these heavily seeded troops rapidly rose to 521/1000/annum in November 1942 and suppression was resumed in all except a few uninfected organizations. This evidence against blanket discontinuation of suppression in highly seeded troops was strengthened in the next few months by unsuccessful attempts to "demalarialize" several heavily infected divisions and regiments (14).³⁹

These experiences were supplemented by similar ones on other islands and the following prerequisites for the discontinuation of suppressive atabrine were developed:

(1) Anopheline breeding must be adequately controlled not only on the occupied portion of the base as a whole, but also in the bivouac area of the individual organization. Furthermore, the routine work and training of the organization was not to include night exposure in malarious territory.

(2) Troops must be unseeded or lightly seeded with malaria. This was determined from the history of previous exposure in malarious areas and by a study of the malaria rates of the organization. Those with a history of a high malaria rate were rarely recommended for discontinuation of atabrine even if this rate fell to low levels under suppression. An index to the degree of seeding in troops with an indefinite history was obtained by discontinuing the drug in a small control group. The trend of the rate was more important than the last rate and it was more hazardous to discontinue atabrine in troops with an upward rather than a downward trend, if other factors were equal.

(3) A record of satisfactory malaria discipline was required.

³⁹ See Graphs VIII and IX, Paper II.

(4) Troops were not removed from atabrine suppression while ground combat was in progress or was threatened; or when they were scheduled for early movement to a combat or malarious area.

The medical officer of an organization in which atabrine was discontinued was advised to make an exception of those men who had had vivax malaria and to continue them on suppressive atabrine. This policy was made official for Army personnel by Medical Circular Letter No. 37, Hq., SoPacBaCom, 10 November 1944, Subject: Treatment of Malaria, which is quoted in part:

"3. Suppressive Therapy After Clinical Attacks. In the future it is desired that those individuals who develop vivax or quartan malaria be placed on suppressive atabrine 0.1 gram daily, total 0.7 gram per week, following treatment of clinical attack. These types of malaria tend to recur repeatedly and suppression should be continued as long as the individual is in this command. The purposes of suppressive medication in this connection are therapy in the broad sense and greater military efficiency. Contrarily, falciparum malaria recurs rarely and there is no need to continue suppressive atabrine in individuals with this type of infection."

H. COMMENT

The huge cost of not being prepared to prevent epidemic disease in the tropics which was evidenced by the occurrence of 200,000 attacks of malaria and the loss of many millions of man days in the South Pacific Area alone is a challenge to make certain that such a situation does not recur. The experiences recounted in these papers indicate that we have the knowledge and the equipment necessary to prevent epidemics of insect-borne disease from jeopardizing military operations in the tropics. The problem is one of personnel trained and prepared and determined to make effective use of such technical know-how and facilities.

Six of the factors which contributed to the control of insect-borne diseases in the South Pacific Area will be discussed as follows: the special organization for this purpose; full support from the high command and from officers of all echelons; a joint Army-Navy-Allied organization; centralized control of policy and personnel coupled with decentralization of operations; integration of survey and control activities and an effective training and education program.

1. A special medical department organization for the control of insect-borne diseases was established by the War Department directives of early 1943 which ordered the creation of malaria survey detachments, malaria control detachments and the use of medical officers as malariologists. Under previous, peace time conditions the control of insect-borne diseases had been adequately accomplished as a joint undertaking by the medical inspector of the surgeon's office and the corps of engineers. But under conditions of active warfare both the medical inspector and the corps of engineers had many other responsibilities of high priority and were also handicapped by the lack of technically qualified personnel for the control of insect-borne disease. The response of the War Department to the problem posed by the early epidemics of malaria was the creation of this special organization whose only responsibility was to control malaria and other insect-borne diseases. The number of highly qualified entomologists, engineers and parasitologists who were brought into this organization was an example

of how highly trained technical specialists cooperated to solve the problems of preventive medicine.

2. The full support which the Malaria and Insect Control Organization received from the military command at all levels was of primary importance. The support of the high command was complete and continuous as soon as the importance of malaria to the military effort was apparent. The fact that malaria alone caused the loss of the use of one to two divisions for more than a year was a constant reminder of the need for control. The support from lower echelons was at first spotty and this was partly due to the lack of information about the seriousness of the problem. The widespread support that was forthcoming once the echelons of command were adequately informed is a bright memory.

3. The joint nature of this Army-Navy-Allied organization for the control of insect-borne diseases was a major factor in its success and was a tribute to the vision of the three surgeons concerned and to the first theatre malaria and epidemic control officer. The great economy of this unified organization was continually apparent in the joint utilization of personnel, equipment and supplies, and was of primary importance during the first eighteen months of the organization when personnel shortages and logistical problems were most acute. The joint use of technically trained personnel was the only possible method of adequately spreading the work of the few qualified individuals to cover the great areas which had to be controlled. Each service was able to complement the other. Thus the army malaria control detachments furnished a majority of sanitary engineers, while the Navy was the chief source of supply officers and of rodent control personnel. Likewise, the army eventually provided the majority of the malariologists while advance groups from the Naval Medical Research Unit #2 furnished specialists who rendered invaluable service in methods of application of DDT, taxonomic investigations and many other problems to which base personnel could not devote sufficient time for thorough investigation. The Navy construction battalions provided the bulk of the heavy earth-moving equipment while the army service of supply was the chief purveyor of motor vehicles, sprayers and other insect control supplies. These supplies were channelled where they were most needed; navy and marine stores were requisitioned for army divisions and vice versa. A careful estimate made in July 1944 indicated that this joint use of personnel effected an economy of at least twenty-five per cent as contrasted with the number which would be required to set up parallel Army and Navy organizations on each base.

4. A theatre policy of centralized responsibility for malaria control coupled with decentralization of operations proved to be an effective answer to an insect control problem on eleven malarious bases scattered over fifteen hundred miles of water and to the added problem of shortages of technically trained personnel and of supplies. A rapidly moving amphibious campaign made it imperative that available personnel be placed where the need was greatest. This was often accomplished by leap-frogging malaria control personnel from a rear base to a forward area. Such mobility was obtained for army personnel by a directive which assigned all malariologists, all survey and all control detachments to

the area headquarters rather than the more usual practice of assigning such personnel to the local military command for which they worked. This malaria control personnel was then attached to base and to division commands as required by existing conditions and circumstances and on the recommendation of the area malaria control headquarters. The area malaria and insect control organization made certain that policies for the prevention of insect-borne diseases were area wide in their application; i.e., directives regarding individual protective measures, the formation of anti-malaria details, the use of suppressive atabrine and similar orders were uniform for all forces.

The responsibility for local insect control operations and for local policies was decentralized to each base. But here again the area pattern of centralized responsibility was repeated and the island malaria and insect control officer was accountable for the insect control work of all forces on the island. This officer, army, navy or allied, acted to coordinate the control activities of all services on the base. He and his technical experts were expected to exercise their own initiative and judgment to solve the local problems. The difficulties of command channels which developed under this unusual system have been discussed. This centralization of responsibility for preventing insect-borne diseases in all forces was obviously an unusual and temporary measure applicable at present only under wartime conditions of great urgency.

5. The effective integration of the activities of malaria survey detachments, of malaria control detachments and of malariologists was in the opinion of the authors the greatest single factor in the success of these groups. This coordination was achieved by an area directive⁴⁰ which placed responsibility for all insect control work on the malariologist. It is to be assumed that this need for coordination had not been fully envisaged when the organization was originally set up, for the malariologist was a staff officer assigned to the surgeon's office and the malaria survey detachment and malaria control detachment were each small autonomous commands. However, experience was clear that this personnel worked efficiently only when they lived and worked together as a team or group. Survey and control were interdependent. Survey work was academic unless translated directly into control activity. Efficient control work required a preliminary survey followed by regular checking of mosquito breeding by the survey detachment. The correlation of these activities with each other, with the overall training program and with other preventive medicine activities was the responsibility of the malariologist. It is probable that equal or better coordination of these army activities would have resulted had these functions been united under a single command.

The improvisation of an Island Malaria Control Headquarters to coordinate insect control activities on large bases has been described. An excellent method of centralizing responsibility for all preventive medicine activities on such large bases with a heavy troop population was put into effect subsequently on Okinawa through the activation of a provisional battalion headquarters⁴¹ with the island

⁴⁰ ComSoPac Serial 002263, 24 October, 1943.

⁴¹ T/O & E.

preventive medicine officer in command. All malariologists, malaria survey detachments, malaria control detachments, medical sanitary companies and rodent control personnel were attached to this battalion. A secondary advantage was the consolidation of reports, requisitions and other administrative details of the small component units. This provisional battalion offered such a satisfactory solution of these administrative problems as to merit more extensive use.

6. The training and education program in insect control work probably paid greater dividends per hour of effort than any other single endeavor. It put many thousands of hands of all degrees of skill to work on insect control, the cumulative effect of which was tremendous. An area training center in malaria and insect control provided excellent training for malariologists and a few others. The inadequacy of this school was in its dependence on the area headquarters staff for its faculty. The school was discontinued when the area malaria control headquarters was moved to the non-malarious theatre headquarters at Noumea, New Caledonia. This school was of such value as to warrant organization with an independent faculty and with a sufficiently mobile organization to move where the greatest number of troops were available for instruction. The other parts of the training and education program deserve no other comment than that they worked smoothly and effectively.

Some additional comments on survey and control activities are pertinent.

7. The need for survey units which are designed for investigational work and having no other duties is adequately discussed in paper III. The need for a parasitological section in these investigational units was amply demonstrated by the contribution of parasitologists in the study of filariasis, schistosomiasis, amoebic dysentery and similar problems. Experience is clear that such investigational work will repay its cost many times over in direct benefit to the military effort.

8. Control activities were handicapped throughout most of the campaign by the long delay between occupancy of a new base and the availability of heavy earth-moving equipment for semi-permanent insect control work. Recommendation was made that suitable earth-moving equipment and position vacancies for operators of this equipment be added to the tables of organization and equipment of medical sanitary companies. The great saving in man days and in troop effectiveness which would result from an early accomplishment of semi-permanent control measures emphasizes the need for some provision whereby such equipment will be directly available to the officer responsible for insect control work.

9. Control measures were characterized by a considerable amount of improvisation in 1942 and 1943 due to the supply shortages. The development of several ingenious substitutes for power sprayers and the manufacture of flumes from empty gasoline drums are examples of this. The use of condemned bangalore torpedoes to replace dynamite resulted from a shortage of the latter explosive and because of the manifest superiority of the bangalore torpedoes for certain types of ditching operation.

Modifications due to the introduction of DDT and special equipment for its

application have been briefly described. Personnel from the South Pacific Area subsequently had an opportunity to utilize airplane application of DDT on a much larger scale on Okinawa, where twenty to forty square miles were sprayed each week. The desirability of both large and small planes for this work was substantiated. It was found that the C-47 type airplane was the best available aircraft for this work because of large load capacity and flying qualities. The presence of a navigator in the crew of the C-47 aided the pilot to fly an exact spray pattern. Moreover, it should be noted that a simple apparatus for the dispersal of DDT solutions by gravity flow with a single outlet under the fuselage gave an adequate coverage at a time when the more elaborate and more efficient spray apparatus described in this paper was not available. The use of DDT solutions on Okinawa exceeded 10,000 gallons daily and required the development of a large scale method of mixing DDT and oil. This was accomplished (22) by using a series of 1200 gallon steel cubes as containers and agitating the DDT mixture with compressed air.

The impregnation of bednets with DDT seems worthy of careful and extensive investigation. The use of this measure in the mounting phase of three divisions from the South Pacific Area indicated the practicability of the procedure. More information is needed about large scale methods of impregnating bednets with DDT, the fire hazard, the duration of insecticidal action and the effect of storage and frequent handling on the latter.

Atabrine as a suppressive drug was of great value but had the obvious defect of hiding the seriousness of the malaria problem and so encouraged laxity in more fundamental methods of control. The great and continued problem of adequate administration of the drug was best solved by giving two doses weekly under careful supervision. The policy of discontinuing atabrine suppression in selected units living in well sanitized areas was consistently followed in the South Pacific Area. It is emphasized again that the control of malaria and of other mosquito-borne diseases was accomplished primarily by insect control.

APPENDIX I

STANDARD OPERATING PROCEDURE FOR CONTROL OF MALARIA AND OTHER INSECT-BORNE DISEASES DURING A COMBAT OPERATION⁴²

This Standard Operating Procedure is merely a sample plan adapted to and used during one operation. All such plans should be flexible. One division malaria control group had 4 general plans calling for various degrees of decentralization, the exact plan and details used to be determined by the particular situation.

1. STATEMENT OF PROBLEM

The target for the operation is an area where a flea, louse and parasite infested native population of several hundred persons per square mile is a seed-bed of

⁴² Variations of this SOP were published three (3) times, in the South Pacific Area Malaria Training Manual No. 2 revised October 1944; in Preventive Medicine Manual No. 2, HUSAF-POA March 1945, and in The Journal of Military Medicine in The Pacific, September 1945, of which the last and simplest is reproduced:

disease. Fly-borne and water-borne intestinal diseases are likely to be the most immediate disease hazards. Native food is contaminated by the use of human feces for fertilizer and should not be eaten. Dengue fever, malaria and mite-borne typhus are potential dangers. Schistosomiasis (blood fluke disease) adds to the risk of drinking or bathing in untreated water.

2. MOUNTING PHASE

a. All personnel will again be trained in individual measures to protect against mosquito and mite bites. Water and food discipline will be emphasized. Fly control measures will be reviewed, particularly the use of DDT solutions and of sodium arsenite solutions to spray corpses. Officers will review the importance of campsite selection to avoid proximity to infected natives and to breeding places of disease carrying insects.

b. Bednets of all personnel will be sprayed with a five (5) per cent solution of DDT in kerosene.

c. Each man will be provided with 2 uniforms and 1 blanket impregnated with dimethylphthalate as outlined in TB Med 121 dated December 1944. Measures in b and c will be carried out as short a time before embarkation as possible.

d. Immunization records will be checked and the necessary booster doses will be given.

e. Suppressives atabrine will be given to all personnel as outlined in TB Med 65, dated July 3, 1944, beginning 3 weeks before D-Day.

f. Each individual will be provided with:

Bar, mosquito or hammock, jungle, complete.....	1
Repellent insect, 2 oz. bottle.....	2
Atabrine tablets, 0.1 Gm.....	30
Insecticide, powder, louse, 2 oz. can.....	1

g. Each organization will be issued 30 days supply of the following items which will be conspicuously marked and carried with the organization so as to be readily available:

Insecticide, powder, louse, 2 oz. can.....	100 per 100 men
Repellent, 2 oz. bottles.....	300 per 100 men
Sprayer, liquid, insecticide, continuous spray, 2 quart.....	1 per 100 men
Sprayer, oil knapsack type.....	1 per 100 men
Diesel oil No. 2, 55 gallon drum with 5% DDT added.....	1 per sprayer oil knapsack type
Atabrine tablets, 0.1 Gm.....	4000 per 100 men
Insecticide, freon-aerosol, 1 lb. dispenser.....	30 per 100 men

3. COMBAT PHASE

a. The malariologist, with the entomologist and parasitologist will provide the surgeon with an insect survey and an estimate of the malaria and insect-borne disease hazard as rapidly as feasible after D-Day, with subsequent estimates as determined by current conditions and needs.

b. Fly control will be done by hand spraying of dead bodies, with 5 per cent DDT solution or with 1 per cent sodium arsenite solution, and by proper care of human waste and garbage, see par. 4.

c. Anti-mosquito measures will be carried out by temporary spray teams as outlined in par. 4. The application of DDT residual effect solution to native dwellings will be emphasized. A single spraying of such buildings with DDT not only will kill all mosquitoes, flies and fleas in the building but will continue to kill them as they come in contact with treated walls for several months.

d. Airplane spraying of DDT will be available about D plus 10. Requests for airplane spraying will be forwarded to the surgeon and will describe the area to be sprayed with an accompanying contour map or grid map. The nature of the insect problem with exact entomological data should be given and an estimate of the need for repeat spraying.

4. TEMPORARY SPRAY TEAMS FOR EACH REGIMENT

a. Each regimental commander will immediately form a temporary spray team comprising 20 men, one man drawn from the insect and rodent control detail of each company. These spray teams will be assisted by 2 technicians, who will be temporarily attached from the Malaria Survey and Control Detachments assigned to Army, Corps or Division. Each regimental spray team will be quartered with its regimental headquarters company for the combat period. The work of these spray teams will be supervised by the Divisional Medical Inspector and by personnel from Malaria and Insect-Borne Disease Control Groups.

b. Duties of These Spray Teams:

In amphibious operations these spray teams will go ashore with the headquarters to which they are attached and begin fly and mosquito control work. Each team will carry out the following measures in the rear of combat lines.

(1) Fly Control. Dead bodies will be sprayed with 5 per cent DDT solution or 1 per cent sodium arsenite solution. Straddle trenches and pit latrines will be sprayed with 5 per cent DDT at the rate of 1 pint per hole twice a week.

(2) Spraying of dwellings and other temporary mosquito control measures in areas of headquarters, medical facilities, supply stations and along communication lines.

c. Equipment of These Spray Teams.

Every man will be equipped with a sprayer for DDT solution. Technicians from Division malaria and insect control groups will carry a dipper for sampling larva populations and preliminary spot maps. Each spray team will carry one week's supply of DDT solution prepared in advance and 3 weeks' supply of DDT powder, dissolving. Each spray team should be equipped with a power sprayer.

d. The Temporary Spray Teams are formed for the period of active operation only. As soon as conditions become stabilized and when designed by the division surgeon, this personnel will return to routine control measures of the company insect and rodent control details.

e. No duties that interfere with their malaria and insect control functions will be assigned to the above personnel.

5. Individual protective measures against mosquito-borne diseases, mite-borne typhus, and schistosomiasis will be carried out by all division personnel as directed.

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MALARIA AND OTHER INSECT-BORNE DISEASES IN THE SOUTH PACIFIC CAMPAIGN

1942-1945

II. EPIDEMIOLOGY OF INSECT BORNE DISEASES IN ARMY TROOPS

W. G. DOWNS,¹ P. A. HARPER² AND E. T. LISANSKY³

Table I and II and Graphs I through XII present data on the incidence of malaria in military personnel in the South Pacific Campaign. Table I and Graphs I through VII pertain to all Army, Navy, Marine and Allied forces on malarious bases. Table II shows the malaria rates for all army personnel on both malarious and non malarious bases; and Graphs VIII through XII show the malaria rates in selected units during their period of duty in the South Pacific Area. The graphs show total rates, primary rates and readmission rates.

The true malaria transmission rate was concealed by suppressive therapy. A low malaria rate was obviously not an index of malaria transmission on a base where all troops were taking suppressive medication. Fortunately the policy of discontinuing suppression in lightly seeded troops provided an indication of the extent of disease transmission. Data for these individual units are not given but a line at the bottom of each graph indicates the percent of troops taking quinacrine hydrochloride (atabrine) suppressive therapy.

The background and other history of these malaria rates is given in papers I, III and IV.

A. EXPLANATION OF GRAPHS AND HISTORY OF INDIVIDUAL BASES

Graph I, Efate, New Hebrides⁴

Efate was the first malarious base to be occupied in the South Pacific, March 1942. Troops were hurriedly sent to this island to build an airfield and forestall the southward advance of the Japanese and were bivouaced in an area which was adjacent to a native camp and where anophelines were numerous. Supplies of antimalarial drugs were inadequate, bed nets were not available for all, and night work was necessary. In April 1942 malaria rates reached 2678 per 1000 per annum. In May about 50% of the troops were directed to take quinine 0.33 grams daily, later increased to 0.66 grams. Even the latter dosage did not provide adequate suppression and in July some units began to take quinacrine hydrochloride 0.4 grams weekly. Malaria control personnel consisting of one officer and three enlisted men arrived on 28 July 1942. Malaria rates decreased

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⁴ Graphs I through XII were originally prepared by Major C. D. Speck, M. C. from data sent monthly to area headquarters by each local malaria control group and first published by Harper et al: Malaria and Epidemic Control in the South Pacific Area.

TABLE I

Malaria rates in all forces in South Pacific—Army, Navy, Marine and Allied

Rate per thousand per annum on malarious bases

BASE	JAN '42		FEB '42		MAR, '42		APR '42		MAY '42		JUNE '42	
	Orig adm	All adm	Orig adm	All adm	Orig adm	All adm	Orig adm	All adm	Orig adm	All adm	Orig adm	All adm
Efate.....							2632	2678	838	982	667	915
	JULY '42		AUG '42		SEPT '42		OCT '42		NOV '42		DEC '42	
Efate.....	354	518	113	209	84	144	135	289	304	520	177	377
Santo.....					36	36	55	59	30	82	137	142
Guadalcanal.....				14		177		1664		1781		972
	JAN '43		FEB '43		MAR '43		APR '43		MAY '43		JUNE '43	
Efate.....	123	290	207	342	163	217	130	179	118	176	72	145
Santo.....	219	255	208	253	150	181	160	191	109	130	62	85
Guadalcanal.....		1169		878		1052		1182		900	396	636
Tulagi.....	229	311	281	409	305	389	352	516	396	593	249	486
Russells.....					281	281	194	204	239	271	283	324
	JULY '43		AUG '43		SEPT '43		OCT '43		NOV '43		DEC '43	
Efate.....	66	128	37	76	58	165	20	92	10	83	18	84
Santo.....	58	98	35	68	20	51	40	83	16	53	17	41
Guadalcanal.....	342	608	142	263	181	287	124	230	91	206	71	149
Tulagi.....	230	417	240	373	214	363	215	366	98	211	97	163
Russells.....	328	395	261	346	146	258	80	137	46	95	57	99
Munda.....		416		329		629		625		504	123	258
Vella-Lavella.....						96		94	42	83	43	104
Bougainville.....									54	58	94	95
	JAN '44		FEB '44		MAR '44		APR '44		MAY '44		JUNE '44	
Efate.....	25	122	14	100	20	79	11	69	10	45	6	37
Santo.....	20	53	7	32	7	28	4	16	3	16	8	19
Guadalcanal.....	130	200	74	126	59	124	50	99	48	95	30	75
Tulagi.....	93	167	91	194	85	177	34	115	27	99	32	107
Russells.....	69	105	129	158	110	189	66	142	71	170	41	104
Munda.....	107	200	57	114	30	65	22	52	20	51	*	
Vella-Lavella.....	67	166	78	160	129	282	133	329	15	54	*	
Bougainville.....	35	119	37	104	37	83	61	103	66	104	*	
Treasury.....	11	12	6	21	6	15	5	26	15	34	*	
Green.....					24	37	29	41	42	62	*	
Emiru.....							58	111	23	46	*	
	JULY '44		AUG '44		SEPT '44		OCT '44		NOV '44		DEC '44	
Efate.....	2	20	4	32	8	30	0	27	0	0	0	0
Santo.....	2	15	1	8	1	5	1	4	0	2	0	3
Guadalcanal.....	33	66	20	58	28	63	22	51	18	43	11	29
Tulagi.....	29	86	9	51	16	58	11	37	14	53	12	26
Russells.....	30	88	12	57	11	45	6	30	10	31	12	29
	JAN '45		FEB '45		MAR '45		APR '45		MAY '45		JUNE '45	
Efate.....	0	0	128	128†	0	0	0	0	0	0	0	0
Santo.....	2	4	4	5	3	4	3	3	7	8	2	4
Guadalcanal.....	18	35	14	25	6	14	8	19	13	19	6	9
Tulagi.....	38	59	15	28	73	99	27	40	54	66	13	26
Russells.....	16	37	18	40	6	15	9	21	5	10	8	8

* Base transferred to South West Pacific Area.

† One case.

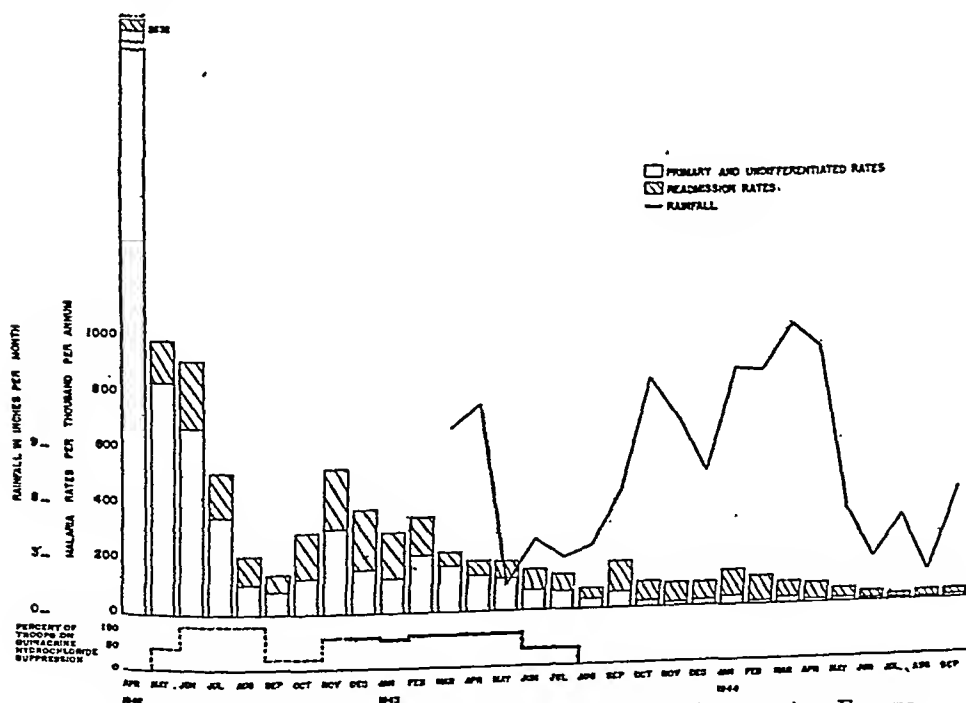
TABLE II*

Malaria rates in army personnel in South Pacific malarious and non-malarious bases

	1942		1943		1944	
	Cases	Rate per 1000 per annum	Cases	Rate per 1000 per annum	Cases	Rate per 1000 per annum
January.....			1818	172	2875	166
February.....			1903	210	2717	152
March.....			2474	253	3444	167
April.....			4596	371	1980	112
May.....			3396	347	2143	124
June.....	290	77	6596	626	1539	84
July.....	320	57	8107	593	632	63
August.....	109	24	7494	696	†	
September.....	184	19	8324	578		
October.....	190	33	7384	528		
November.....	238	38	5414	400		
December.....	480	67	3779	252		
Total.....	1811	38	61,285	427		

* Data supplied by tropical Disease Control Division, office of Surgeon General; compiled from 86 AB reports.

† The South Pacific Area became part of the Pacific Ocean Area, later called the Middle Pacific Area, at this time.



GRAPH I. EFATE. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES.
(See Table I for data from November 1944 through June 1945)

rapidly from the April peak because of the initiation of insect control measures, suppressive therapy and the onset of the dry season. Quinacrine was supplied to natives for suppression of parasitemia; native huts near troops were sprayed daily with a pyrethrum spray; and finally, nearly a year after the original epidemic, the largest native labor camp, "Riserville", was relocated a safe distance from troops. Antimosquito work was well organized by early 1943. Heavy equipment for semipermanent malaria control projects was not available until 16 months after occupation.

The low total rate of 144 per 1000 per annum in September 1942 induced Army and Navy units to discontinue suppressive therapy. When the November rate rose to 520 per 1000 per annum, suppressive therapy was resumed in all except certain Navy personnel who were lightly seeded and living in screened quarters. Many of the heavily seeded units left Efate in 1943 and the remaining troops were withdrawn from poorly controlled areas. Quinacrine suppression was discontinued in all troops in mid-1943 and the subsequent low rates are unmodified by drug suppression.

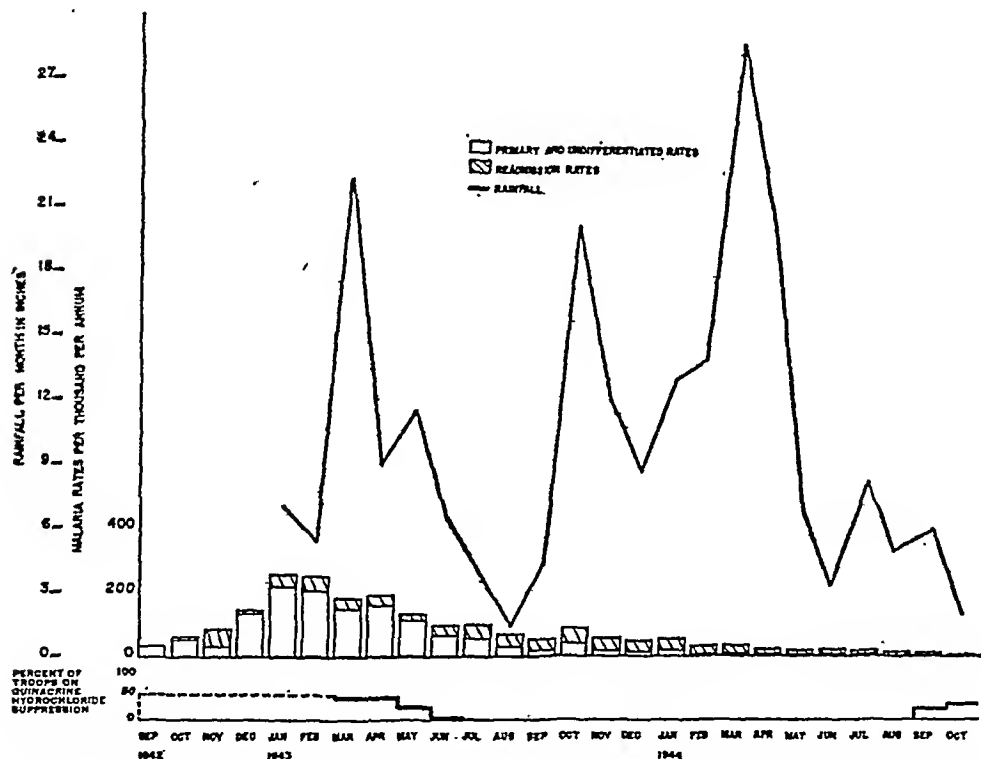
Graph II, Espiritu Santo

The unopposed occupation of this base was made at the beginning of the dry season in May 1942. There was minimal mosquito breeding near the original campsites and until September of that year no cases of malaria were reported even though drug suppression was not practiced. In September, October and November 1942 incoming troops were camped near heavily seeded natives in areas of anopheline breeding. Quinacrine suppression was ordered for the more exposed personnel and rates did not rise above 256 per 1000 per annum during the rainy season of early 1943. A Base Malaria Control Group consisting of 2 officers and 8 enlisted men was established in September 1942. Troop concentrations were kept separated as far as possible from natives. Natives were started on suppressive therapy in early 1943, and native huts were sprayed regularly with pyrethrum insecticide from early 1943 to mid-1944, when DDT residual spraying was started. By mid-1943 an extensive and effective malaria control program consisting of larvicidal and semi-permanent control measures was in operation, and following this it was unusual to find adult anophelines in the main occupied areas. In July 1943 suppressive therapy was discontinued for troops in well controlled localities. Despite adequate control in the main occupied area, outlying regions on the base continued to be highly malarious and troops on outpost duty became heavily infected. Rates for Espiritu Santo are not comparable with rates from other South Pacific malarious bases because only cases occurring in local units and of local origin were considered in the computations. An undetermined small amount of malaria which actually was contracted on Espiritu Santo plus a larger amount of malaria occurring in transient personnel and representing malaria contracted elsewhere, was therefore never reported on this base. On other bases, all cases of malaria were usually included in the determination of local malaria rates, regardless of the origin of the infection. Excepted from this policy were those patients who developed malaria on

a forward base and who were temporarily housed in hospitals on successive rear bases during evacuation to a non-malarious island.

Graph III, Guadalcanal

The campaign for the Solomon Islands began in August 1942 with the invasion of Guadalcanal by Marine units. These forces were supplemented in October by Army units. Antimalaria supplies were at first unavailable or inadequate, and no significant amount of mosquito control work was accomplished. There were only a few cases of malaria in August and September. However, conditions

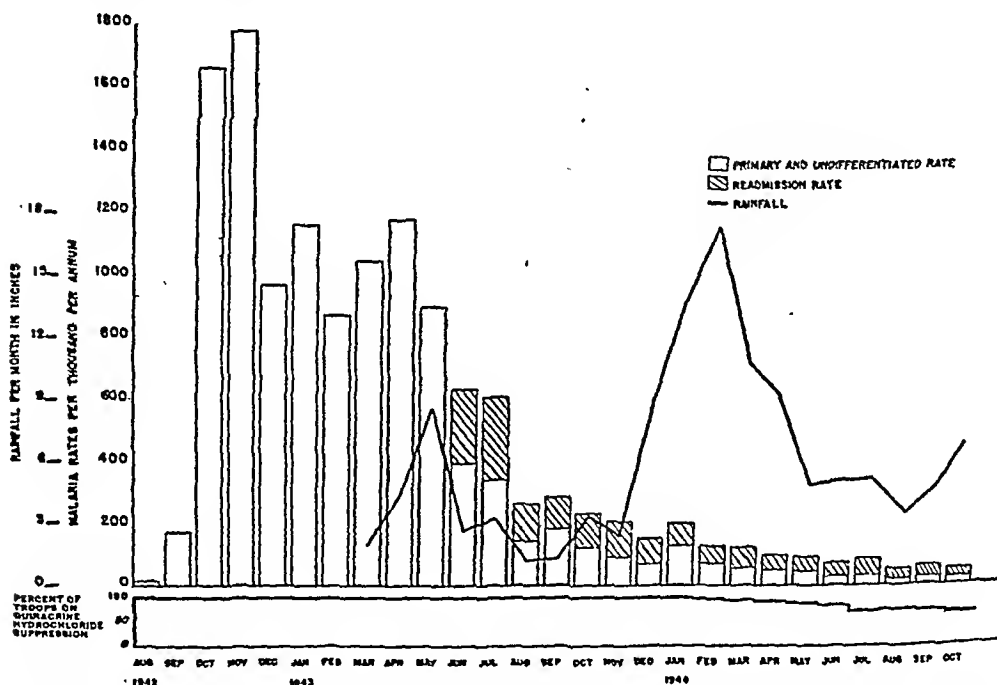


GRAPH II. ESPIRITU SANTO. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES. (Not comparable with rates of other bases. See text). (See Table I for data from November 1944 through June 1945)

were ideal for the breeding of enormous numbers of mosquitoes during this period. The flat alluvial plain of the contested area was traversed by more than 40 rivers and small streams. There were over 50 lagoons. The soil was a heavy clay which was difficult to drain. Mosquito breeding areas were increased by the multitude of shell holes and fox holes, and ruts caused by heavy vehicles. It has been estimated that "man made" breeding places accounted for over 50% of the mosquito breeding during the first year of occupation. The disease became epidemic in November with a peak case rate of 1800 per 1000 per annum in this month. Epidemic conditions prevailed for at least 9 months. It is probable that of the estimated 100,000 cases of malaria contracted in the South

Pacific area more than three-fifths of the total number were contracted on Guadalcanal, largely during the period from November 1942 to August 1943. The malaria rate decreased to less than 200 per 1000 per annum by December 1943 and continued to fall in spite of a steady increase in the percent of troops released from suppressive medication.

A Base Malaria Control Group, (initially composed of Navy personnel, later becoming a combined service group) of 2 officers and 8 enlisted men was established in November 1942. This organization in time expanded greatly, and the base received by far the largest amount of antimalaria effort of any island in the area. Control work was well advanced by the latter part of 1943 and thence-

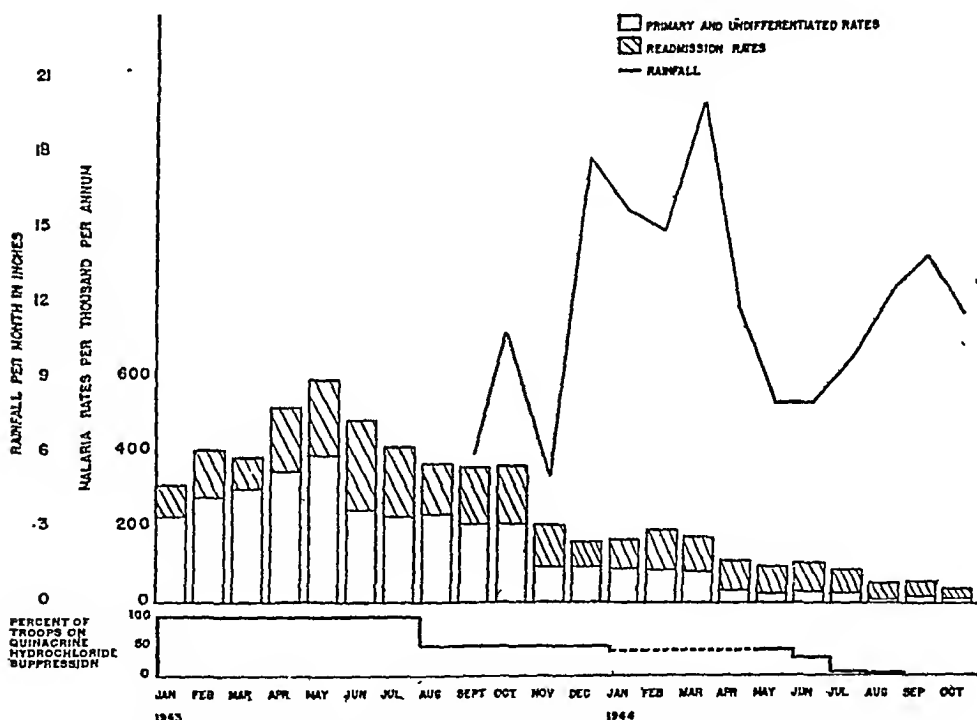


GRAPH III. GUADALCANAL. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES. (See Table I for data from November 1944 through June 1945)

forth adult anophelines were difficult to find except in a few marginal areas and during sporadic infestations in the central areas.

Factors contributing to the control of epidemic malaria on Guadalcanal are so inextricably intertwined that it is impossible to examine them individually and to assess accurately the relative importance of each. The most important factor was the extensive malaria control work, both larvicidal and drainage, which was accomplished. Other important factors included quinacrine suppressive therapy as an aid in lowering rates in heavily seeded troops; carefully supervised handling of natives, including administration of suppressive treatment and mass therapy, spraying of native dwellings, the removal of labor camps from troop areas by August 1944; the improvement of living conditions for troops, including an eventual great increase in the number of screened dwellings; and extensive troop education in personal malaria preventive measures.

A significant development in November 1943 was the establishment of a troop bivouac area of about 90 square miles, where mosquito control was continually exercised. No organization bivouaced outside this area without special permission. Troops staging on this base were given approved locations for their temporary camps, thus eliminating a very prominent source of malaria during the early Solomon's campaign. During this early period troops who had staged on Guadalcanal in uncontrolled areas for only a few days and then moved on to another base, would often have severe malaria outbreaks largely traceable to their exposure on Guadalcanal.



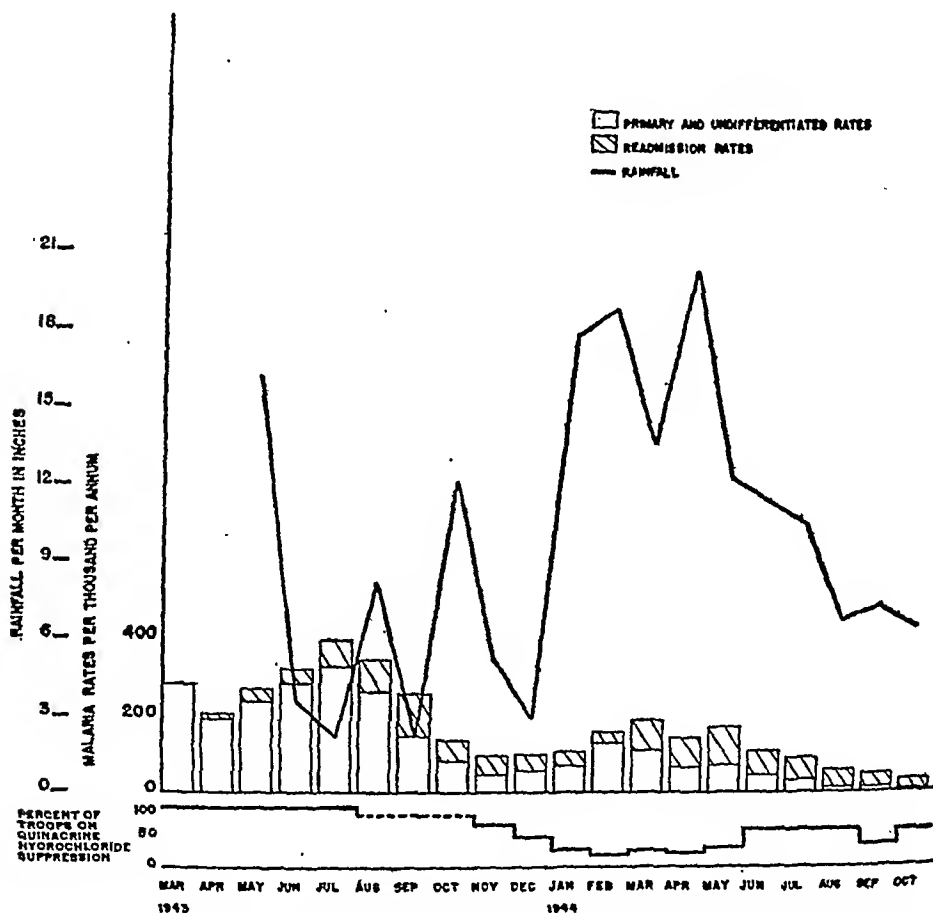
GRAPH IV. TULAGI-FLORIDA. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES. (See Table I for data from November 1944 through June 1945)

In February 1944, the discontinuance of suppressive therapy was begun in lightly seeded units. By October 1944 about 26% of the troops had been allowed to discontinue quinacrine and the island total malaria rate was only 51 per 1000 per annum. The malaria rate continued to decrease and by June 1945 over 75% of troops were released from suppression.

Graph IV, Tulagi-Florida

American forces landed in the Florida group of islands against brief but spirited Japanese opposition in August 1942. Small groups of troops were widely scattered and antimalaria supplies were inadequate. No rates are available for the period prior to January 1943, at which time malaria control activities were

instituted by a Navy malaria control group. In one organization of 237 men the malaria rate in December 1942 reached 2004 per 1000 per annum while the group was supposedly taking 0.4 grams of quinacrine weekly. The maximum recorded rate for this entire group of small bases was 593 per 1000 per annum in May 1943. Most of the cases occurred in a few units in poorly controlled areas and therefore drug suppression was discontinued in about half the troops in August



GRAPH V. RUSSELLS. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES.
(See Table I for data from November 1944 through June 1945)

1943. In September 1944 quinacrine was discontinued in all personnel when the total rate on this base was 58 per 1000 per annum.

Effective control work played a major part in reducing epidemic malaria on these small bases. The work was made difficult and complex by the scattered position of the small groups. Several of the troop units and areas were accessible only by boat.

Graph V, Russell Islands

The 43rd Division made initial landings on Banika Island in the Russell group in late February 1943. This division had been previously seeded with malaria

on Guadalcanal and the monthly rate for March was 281 per 1000 per annum. There were neither natives nor Japanese on Banika or Pavuvu, the two main islands occupied. Mosquito breeding sites were numerous on both islands, and hundreds of small round ponds, about thirty feet in diameter, were scattered throughout the coconut plantations.

A base Malaria Control Unit consisting of one officer and three enlisted men arrived on March 3, 1943, to find a serious outbreak of *P. falciparum* malaria among troops who had been resident less than two weeks on the base, and incoming units from Guadalcanal showed the same picture. Rates of 200-400 per 1000 per annum continued for many months, in spite of quinacrine suppressive therapy of 0.4 grams per week which was poorly supervised.

Although no natives were originally present on Banika Island, 600 labor corps natives arrived in late 1943. The area occupied by them was kept under adequate control; huts were sprayed, suppressive therapy was administered and natives were restricted to their own camp area after 1800 hours.

For many months control measures were limited to larvicidal work, and troop areas were in large part well controlled. No significant amount of heavy equipment was used for more than a year after occupation; during 1944 heavy equipment projects were about 90% completed.

Discontinuance of suppressive therapy in selected lightly seeded units began in August 1943, and permitted a malaria peak of 189 per 1000 per annum in March 1944. By October 1944, improvement of control work with an increase in the proportion of troops taking quinacrine produced a decline in the rate to 30 per 1000 per annum.

Graph VI, Munda, New Georgia

The 43rd Division invaded Rendova Island in the New Georgia group on July 1, 1943. Small groups also landed at Wickham Anchorage, at Segi and at Viru Harbor, all of these being on New Georgia. The main invasion of New Georgia by the 43rd Division, later joined by the 37th and 25th Divisions took place in the Munda area in mid-July. A Malaria Control Group landed on Rendova on July 11, 1943 and moved to the main base at Munda in August.

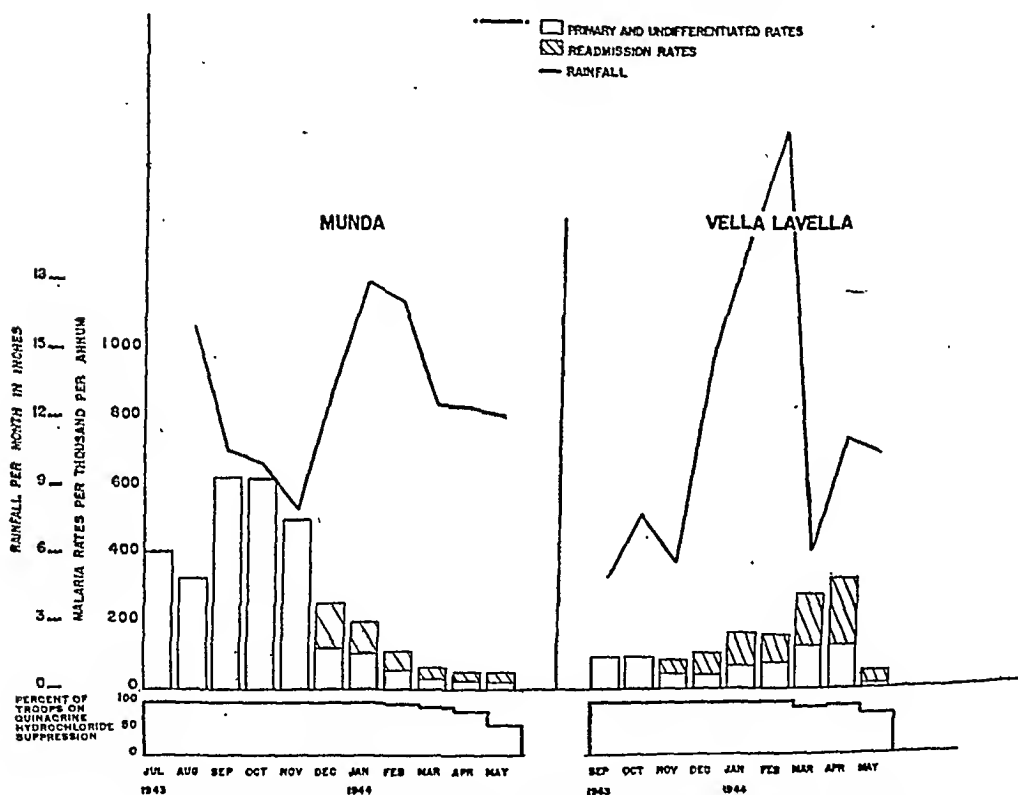
Heavy combat during the first two months resulted in many shell and bomb craters, ruts and similar sources of man made malaria. The seed bed consisted of Japs and Allied troops previously infected on other bases. All Allied troops took quinacrine suppression, 0.4 grams per week, poorly supervised. For the first time in South Pacific campaigns, there was a small supply of insect repellent and aerosol bombs.

Rendova presented a serious anopheline breeding problem. It was temporarily controlled for a month and then abandoned. New Georgia presented difficulties because of innumerable small breeding areas, bomb craters and ruts, scattered over a wide area. Early control efforts with insufficient personnel, equipment and transportation could only partially cope with the problem, but no serious difficulties were encountered when more personnel and equipment became available in October 1943.

Control groups were also established at Segi and at Ondonga, on New Georgia, to cope with insect-borne diseases in small numbers of troops. Local camp-site control was the main effort on such islands as Baangam, Arundel, Roviana, Sasavelo, Bau, Kokorana, Barauna and Kolombangara.

A native labor camp was set up on an offshore island and presented no problem.

Malaria rates rose to 629 per 1000 per annum in September 1943, with the heavily seeded 25th Division showing a rate of 1000-1500 per 1000 per annum. Shortly after this peak a large proportion of heavily seeded units left the base, and by May 1944 rates had dropped to 51 per 1000 per annum.



GRAPH VI. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES
(Bases transferred to SWPA 15 June 1944)

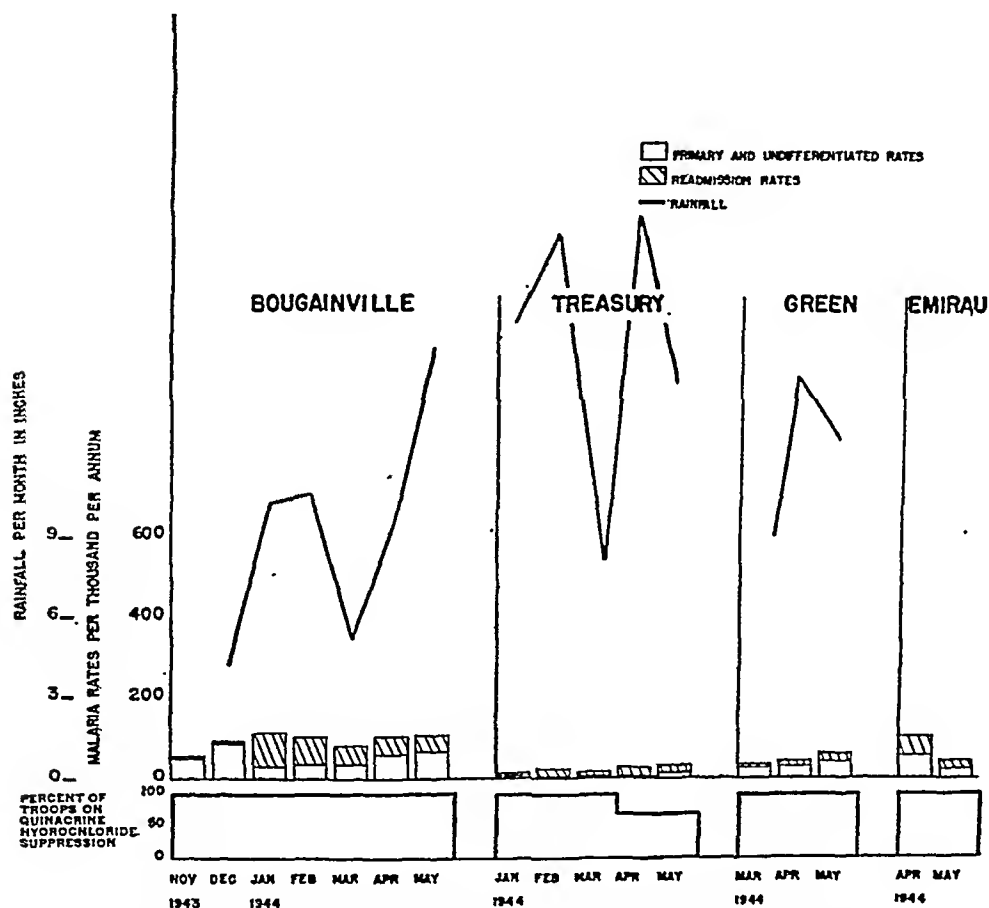
Graph VI, Vella Lavella

Vella Lavella was occupied in August 1943 by New Zealand troops aided by a small American force. The malaria control unit attached to the 3rd New Zealand Division, consisting of 4 officers and 33 enlisted men, arrived with the division. In addition, a Navy control group was also present, working in areas occupied by U. S. troops. Most of the malaria cases seen on this base occurred in a single Marine Defense Battalion which had been heavily seeded on Efate in 1942. By April 1944 the rate in this battalion was 964 per 1000 per annum but with improved drug suppression fell to 114 in May, as compared with an island rate of 54 per 1000 per annum. Forces on Vella Lavella were dispersed over a

long stretch of coast line, and the low rates in the New Zealand Division (previously unseeded) are a tribute to their malaria discipline and the efficient larviciding program maintained.

Graph VII, Treasury Island

This small island was occupied by New Zealand troops in October 1943 and a section of the New Zealand malaria control group proceeded there. A small



GRAPH VII. MALARIA RATES PER THOUSAND PER ANNUM, ALL FORCES
(Bases transferred to SWPA 15 June 1944)

Navy control group also was established on Treasury. All natives were evacuated from Treasury to Mono. An early larvicidal program was followed in early 1944 by drainage of the few swamps present.

Graph VII, Bougainville

Landings were made at Empress Augusta Bay, an almost uninhabited area, in November 1943. Fully organized malaria control groups went in with the combat divisions; a Navy Malaria Control Group with the 3rd Marine Division

and an Army Malaria Control Group with the Americal and the 37th Divisions. In January 1944 a Base Malaria Control Group was established for service troops. The occupied area was so difficult to approach from other Jap held parts of the island that the main ground combat did not take place until March 1944, although the early period of Marine occupation was characterized by bitter fighting in dense jungle and swamp. There were extensive swamps near the beach and our troops established camps at some distance from the beach, where the underlying sandy soil proved to be very easy to drain. Control work was started early and malaria never became a serious problem. The peak rate of 119 per 1000 per annum was recorded in January 1944. Many of the troops were previously seeded. Quinacrine suppressive therapy was universal, 0.6 gram per week, and while far from perfectly supervised, was better observed than during any previous major combat period. Adequate supplies of insect repellent and aerosol bombs were available, and use, at least of aerosol bombs, was widespread.

A large group of natives was quartered almost in the middle of the troop concentration, but in an area which was carefully supervised and rigidly controlled.

Minor outbreaks of malaria occurred in troops on perimeter defense, and in units making sorties into Jap held territory. It was interesting to observe a high incidence of *P. falciparum* infections in these outbreaks. Most of the cases occurring in controlled areas were *P. vivax*, and the history of these cases indicated that the majority of them were relapses of infections acquired elsewhere.

Mosquito control measures were well advanced and adult anophelines were difficult to find in the occupied area after the first quarter of 1944.

Graph VII, Green Island

This small island was occupied in February 1944 by New Zealand troops, with attached malaria control personnel. Later, a Base Malaria Control Group was established. There were few anopheline breeding sites and 90% of the natives were removed to another island. Effective control work was instituted early and malaria never became a problem.

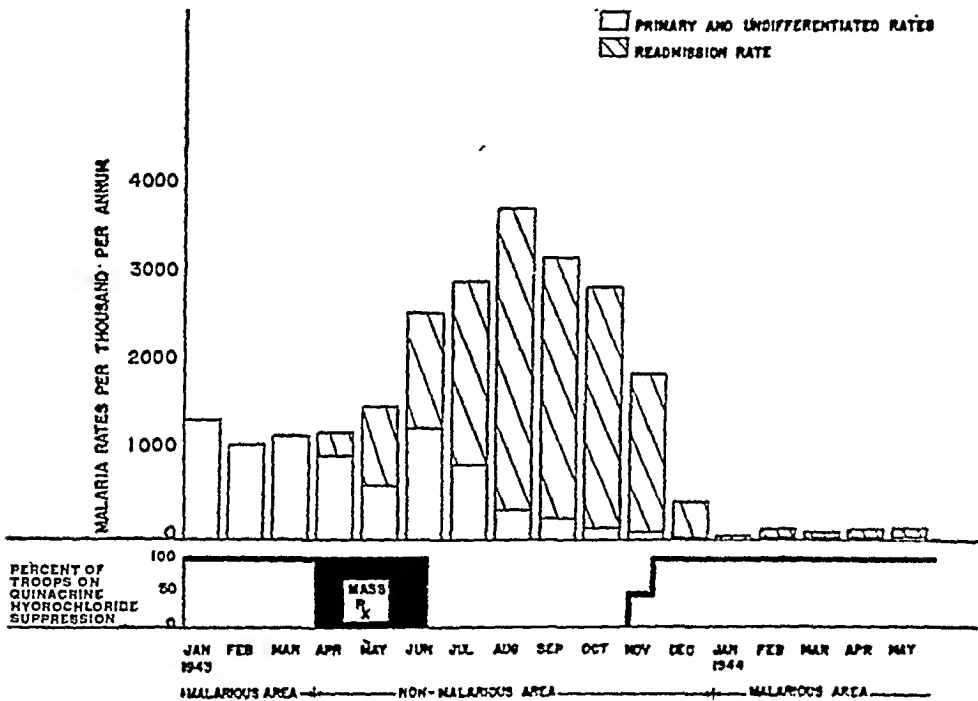
Graph VII, Emirau Island

This small island of the St. Matthias group was occupied in March 1944. A Malaria Control Group accompanied occupying forces and a very effective control program was instituted early. Even heavy equipment drainage projects were completed in three months. 220 natives on this island were relocated on a neighboring island. Although extensive anopheline breeding areas existed on Emirau, early larvicidal work followed by drainage of all swamps prevented malaria from becoming epidemic.

In June 1944 the bases of New Georgia, Vella Lavella, Treasury, Bougainville, Green and Emirau were transferred to the South West Pacific theater and malaria statistics are not available for those bases after that date. However, by this time all of these bases had well organized malaria control organizations and epidemic malaria had ceased to exist among troops.

Non-Malarious Bases

Malaria control personnel in New Zealand, New Caledonia, Samoa and Bora Bora aided in demalarialization programs carried out in troops sent to these areas for rest and rehabilitation. Several of the hospitals in New Zealand, Fiji and New Caledonia also assisted or in some cases carried a major part of this program. Laboratory diagnostic centers were established and statistical data was accumulated on malaria in the troops. In Samoa and Bora Bora the malaria control organizations aided and supervised an antimosquito program directed against the vector of filariasis.



GRAPH VIII. AMERICAN INFANTRY DIVISION. MALARIA RATES PER THOUSAND PER ANNUM IN RELATION TO EXPOSURE AND TO THERAPY
Scale differs from Graphs I-VII

B. MALARIA EXPERIENCE OF CERTAIN ARMY UNITS

Graph VIII, Americal Division

This division moved by echelons to Guadalcanal in October. November and December 1942. In March 1943 it was transferred to Fiji for "demalarialization" and rehabilitation. While on Guadalcanal there was little or no field control, no repellents or aerosol bombs, and very little malaria discipline. The troops lived and fought in areas where malaria was epidemic. Quinacrine suppressive therapy, 0.4 gram per week, was prescribed, but the extent of its use has been questioned and cannot now be determined. The monthly malaria rates were as high as 1358 per 1000 per annum while in the combat zone. After evacua-

tion to Fiji, mass treatment with quinacrine and plasmochin was given to the entire division from April to June. Plasmochin was discontinued in those who did not tolerate it and additional quinacrine was given this group. After this mass therapy, no antimalarial drugs were administered except to those having clinical attacks of malaria. With the discontinuance of drugs and in spite of the previously administered mass therapy, the monthly rate rose in August to a peak of 3760 per 1000 per annum and in October was still at the high level of 2880 per 1000 per annum. On November 1, when the division was alerted for return to a combat area, quinacrine suppressive therapy was gradually resumed, 0.4 gram per week, and increased to 0.6 gram per week in mid-December. The malaria rate fell to 43 per 1000 per annum in January, a dramatic demonstration of the suppressive power of quinacrine. After 5 months of combat duty on Bougainville the total rate for May 1944 was only 112 per 1000 per annum. A Malaria Control Group, (a malariologist, a malaria survey detachment and a malaria control detachment) was attached to this division in December 1943.

The changes in malaria parasite species partition which occurred after this division left the malarious area was interesting. *P. falciparum* comprised more than fifty per cent of all malaria reported on Guadalcanal during January, February and March 1943, and *P. vivax* comprised about twenty-five per cent of cases, Figure I, Paper IV. This predominance of *P. falciparum* continued for a few weeks after arrival in Fiji and was then rapidly reversed and *P. vivax* constituted practically all subsequent cases.

A study of the effects of malaria on this division is given by Tumulty et al. (1).

Graph IX, 147th Infantry Regiment

A presentation of the early history and attempted "demalarialization" of this regiment in Upolu, British Samoa, has been published recently (2).

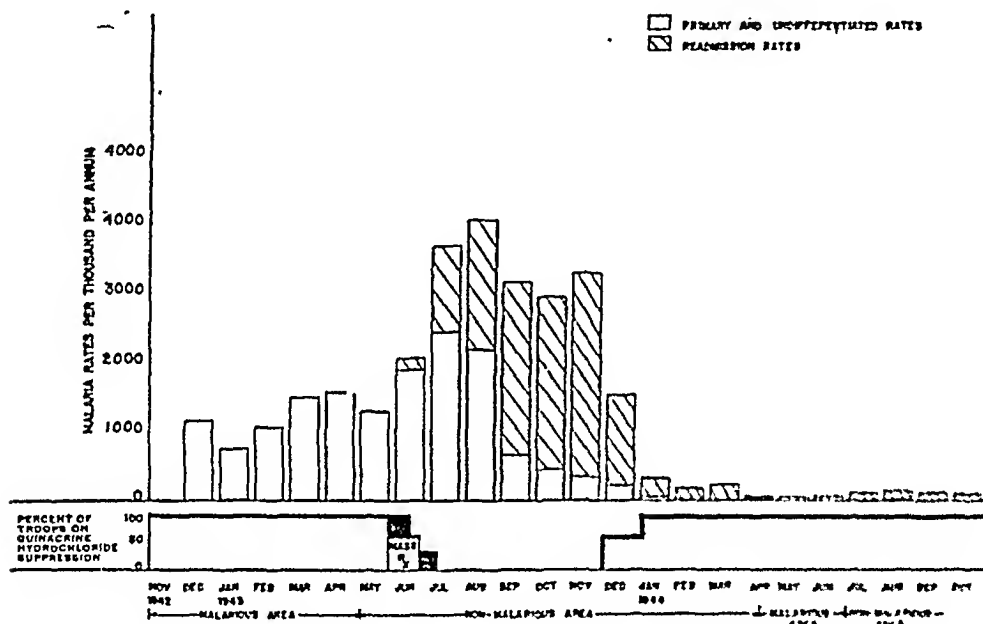
The 1st Battalion of this regiment disembarked at Guadalcanal on 4 November 1942, the 3rd Battalion on 29 November 1942, and the 2nd Battalion on 7 February 1943. The entire group departed from Guadalcanal for Samoa in May 1943. Except for the 1st Battalion which used quinine during November, suppression was with quinacrine 0.4 gram per week, but was not closely supervised until late March.

The morning sick reports while on Guadalcanal showed malaria in 48 percent of the regiment, and monthly rates for the disease during this period reached a peak of 1558 per 1000 per annum in April, although most of the diagnoses were unconfirmed by blood smear examination.

"Demalarialization" was begun in Samoa in May 1943. Four plans were used: (1) quinacrine mass therapy immediately, (2) quinacrine mass therapy after a ten day interval of no medication, (3) mass treatment with both quinacrine and plasmochin, and (4) no mass therapy. Suppressive medication was then discontinued in all of these groups and clinical cases were treated as they occurred. The termination of mass therapy was staggered so that while peak rates in the different groups exceeded 14,000 per 1000 per annum, yet the rates of the regiment as a whole during this period did not exceed 4090 per 1000 per annum.

The 2nd Battalion was on Wallis Island from October 1943 to January 1944, when all three battalions were moved to New Caledonia. Suppressive quinacrine was reinstituted on 26 November 1943, 0.4 gram per week, and later increased to 0.6 gram. In November, before suppression was reinstituted, the rate was 3290 per 1000 per annum. In January, after two months on quinacrine, the total rate had dropped to 334 per 1000 per annum. In March the regiment was sent to Emiru, returning in June to New Caledonia.

A reversal of parasite species partition from *P. falciparum* to *P. vivax* occurred in this regiment after leaving Guadalcanal and was similar but more marked than that described for the Americal Division.



GRAPH IX. 147TH INFANTRY REGIMENT. MALARIA RATES PER THOUSAND PER ANNUM IN RELATION TO EXPOSURE AND TO THERAPY
Scale differs from Graphs I-VII

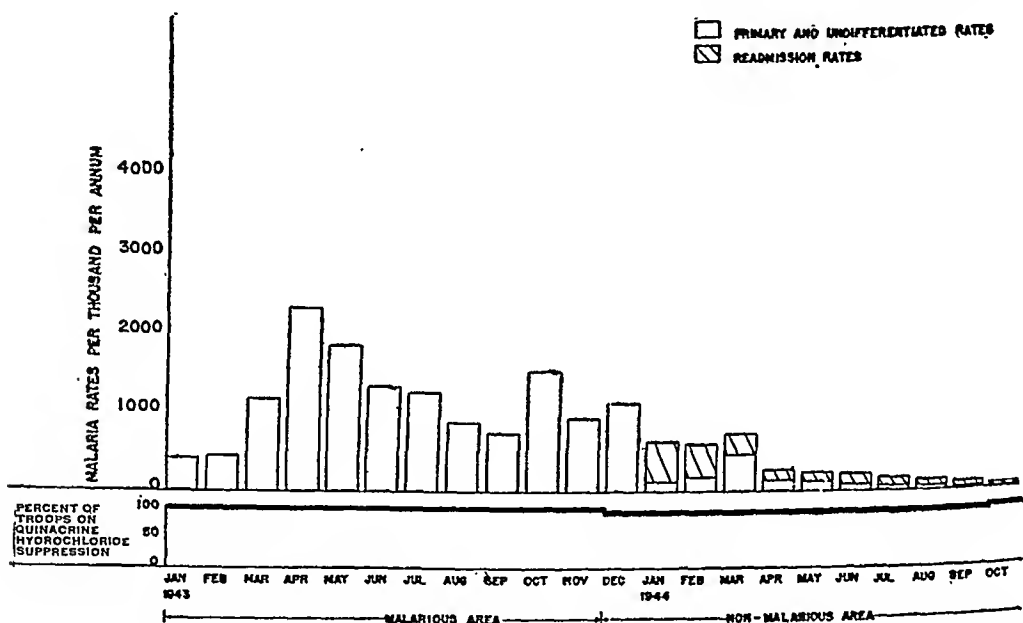
Graph X, 25th Division

This division arrived on Guadalcanal from Hawaii in late December 1942 and took part in the latter part of the campaign for that island. The personnel soon became heavily infected, with a peak rate of 2335 per 1000 per annum in April 1943, despite quinacrine suppressive therapy, 0.4 gram per week, which was not adhered to strictly. The division participated in combat on New Georgia and Vella Lavella from late July to October 1943. In October the rate was 1550 per 1000 per annum. In December the division went to New Zealand and later to New Caledonia. Quinacrine was discontinued in a small control group to determine the amount of seeding. After four weeks, the rate in this group was 2901 per 1000 per annum. Repeated efforts to improve quinacrine administration were associated with a progressive decline in rates to 41 per 1000 per annum in October 1944.

The continuation of suppression in this heavily seeded division during their rehabilitation period in non-malarious areas represented a change in policy from that followed with earlier divisions, the First and Second Marine Divisions, the Americal Division and the 147th Infantry Regiment. The repeated relapses and continued high rates in these divisions in which quinacrine was discontinued clarified the need for continuous suppression if troops were to be rehabilitated promptly for return to combat duty.

Graph XI, 37th Division

This division moved to Guadalcanal in March 1943 and was bivouaced in a relatively well controlled portion of the island, where it instituted an early



GRAPH X. 25TH INFANTRY DIVISION. MALARIA RATES PER THOUSAND PER ANNUM IN RELATION TO EXPOSURE AND TO THERAPY
 Scale differs from Graphs I-VII

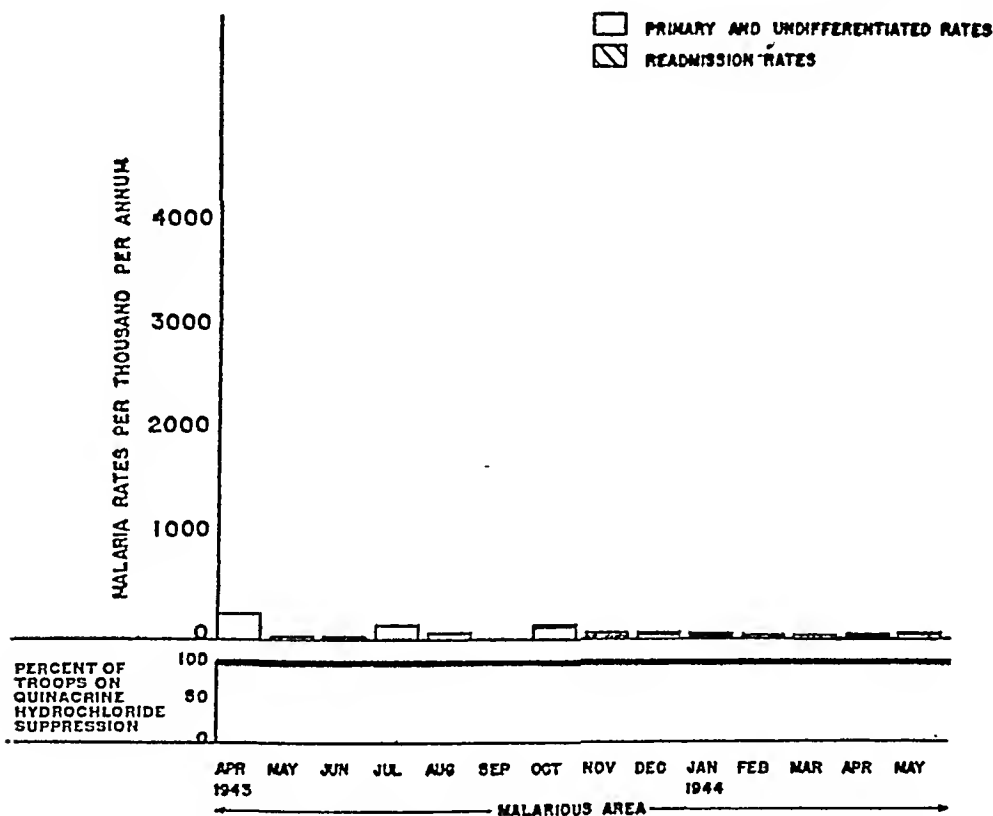
and effective program of larviciding and enforced use of individual anti-malaria protective measures. From late July to September 1943 the division participated in the New Georgia campaign, then returned to Guadalcanal for rehabilitation and restaging. Quinacrine taken by roster 0.4 gram per week was increased to 0.6 gram per week in mid-October 1943. The division was then sent to Bougainville in November 1943 and remained under combat conditions at that base for nearly a year.

Total rates remained low, explained in part by good fortune in having a relatively well controlled area on Guadalcanal at the time epidemic malaria existed there. Credit is due this division for early recognition of the malaria hazard, excellent individual protection, a well supervised program of suppression, and an adequate organization of troop unit anti-malaria details.

A malaria control group was attached to the division in October 1943, the first time that such a group was used for an Army division in the South Pacific theatre.

Graph XII, 43rd Division

This division was moderately heavily seeded with malaria in the Koli Point area of Guadalcanal in February 1943, while enroute to the Russell Islands, and continued in malarious areas on the Russell Islands and New Georgia, through



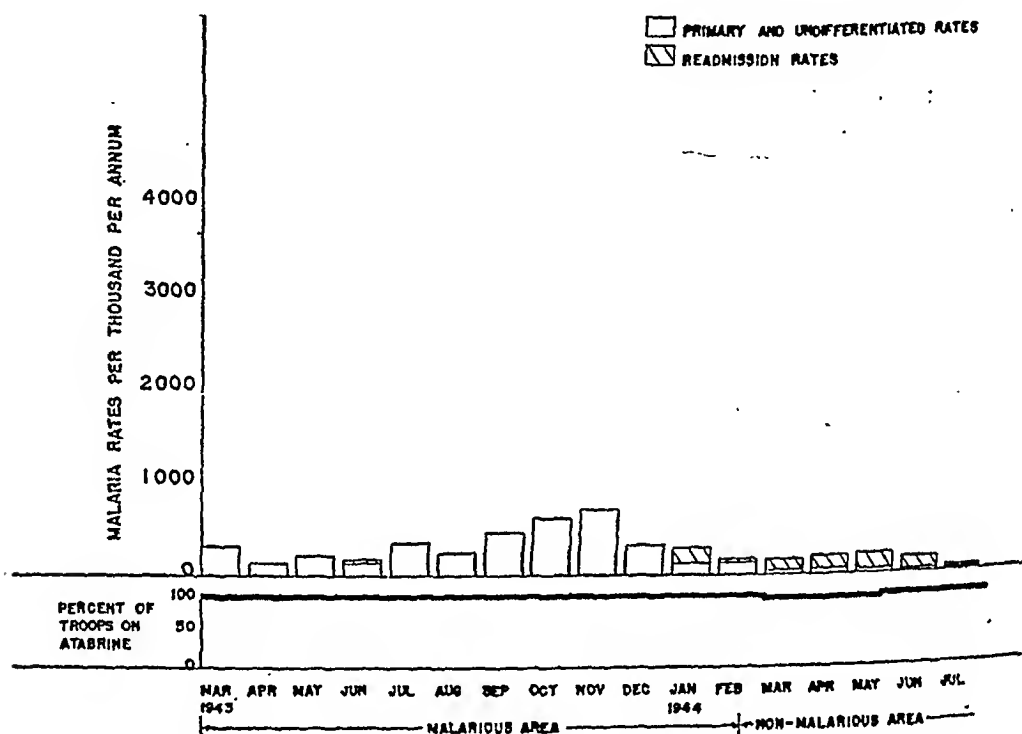
GRAPH XI. 37TH INFANTRY DIVISION. MALARIA RATES PER THOUSAND PER ANNUM IN RELATION TO EXPOSURE AND TO THERAPY
Scale differs from Graphs I-VII

December 1943. During February and March 1944 in New Zealand, where the division had gone for rehabilitation, quinacrine was discontinued in a small control group to determine the amount of seeding. In one company of this group the rate rose to 2025 per 1000 per annum, approximately half the peak rate of the Americal Division. The remainder of the division continued on quinacrine suppressive therapy, 0.6 gram per week, with monthly rates not exceeding 235 per 1000 per annum. By July 1944 the rate was 64 per 1000 per annum. A Malaria Control Group was attached to this division in May 1944.

C. DENGUE FEVER

Epidemics of dengue fever occurred in 1943 on Fiji, New Caledonia, Efate, Espiritu Santo and Tulagi-Florida. The disease was endemic in the local populations. The vectors were domestic mosquitoes which are discussed in Paper III, mainly *Aedes Aegypti*.

Table III shows the uncorrected army rates of this disease for 1943 and 1944 in New Caledonia and Espiritu Santo. In New Caledonia the epidemic of 1943 was localized to the Noumea area but rates were calculated on island strengths.



GRAPH XII. 43RD INFANTRY DIVISION. MALARIA RATES PER THOUSAND PER ANNUM IN RELATION TO EXPOSURE AND TO THERAPY¹
Scale differs from Graphs I-VII

¹Total army malaria rates are given for part of the period when the 43rd Division was on the Russells Islands at which time separate rates for this division were not available.

The problem was complicated by the presence of a large civil population. A medieval plumbing system with open drains and inadequate run-off afforded ideal breeding sites. Many cellars contained water thruout the rainy season. *Aedes Aegypti* larvae were found in flower-pots in the town and cemetery, in laundry tubs and other water containers and in a shipment of rubber tire casings. A house to house inspection and educational program was instituted in cooperation with the French Director of Public Health. Educational material and posters were printed in both French and English.

The 1943 epidemic on Espiritu Santo affected approximately 25 percent of the military population with an estimated loss of 80,000 man days (Paper I). For several months the eradication of breeding sites of domestic mosquitoes

became more important than anopheline control and occupied a large share of the energy of the base malaria and insect control organization. No cases occurred in 1944 because of adequate mosquito control, even though there were numerous newly arrived and susceptible military populations.

There was no significant outbreak of this disease in 1944 in the military on either Efate or Tulagi-Florida.

D. OTHER INSECT-BORNE DISEASES

Over 70 cases of tsutsugamuchi fever occurred on Bougainville and 3 on Espiritu Santo. About 2,000 cases of filariasis occurred in army personnel, contracted chiefly in the islands of Polynesia. References are given in Paper I.

Great epidemics of enteritis occurred in troops during combat operations on Guadalcanal, Tulagi and Munda, New Georgia. Careful bacteriological studies

TABLE III
*Comparison of 1943 and 1944 dengue epidemics**
Army rates per 1000 per annum

	ESPIRITU SANTO		NEW CALEDONIA	
	1943	1944	1943	1944
January.....	—	0	0.4	1
February.....	441	0	65	19
March.....	1095	0	186	60
April.....	1713	0	645	54
May.....	1531	0	317	18
June.....	909	0	66	12
July.....	245	0	30	—
August.....	82	0	3	—

* From Newsletter No. 9 Hq. Malaria and Epidemic Control, South Pacific Area, March 1944 and from Harper et al., Malaria and Epidemic Control South Pacific Area 1942-1944, p. 279b.

were not done. Many observers were impressed with the association between these epidemics and the enormous fly populations which developed in dead bodies, and in exposed feces and garbage. The outbreaks subsided rapidly after the eradication of the fly populations and without any significant change in kitchen or mess sanitation. In a subsequent operation on Okinawa strenuous efforts were successful in preventing the development of significant fly populations. There was only a rare case of enteritis among troops in this latter operation during the first 75 days when combat was severe. No conclusions are drawn from this observation.

E. COMMENT

The epidemiological experiences of military forces in the South Pacific area during the course of three years, involving establishment of many bases in malarious areas, often during combat, are difficult to interpret or to summarize ade-

quately. An immense problem confronted malaria workers on military bases in the New Hebrides and Solomon Islands in late 1942 and early 1943. Malaria was epidemic, trained personnel were few, equipment for control work was inadequate, and stocks of drugs for treatment were low. All efforts were directed to the control of malaria as quickly as possible. The press of immediate control work precluded careful epidemiological studies on the disease, and its vectors. Detailed studies which were conducted later when trained personnel became available are incomplete, since they were undertaken during conditions approximating low grade endemicity rather than epidemic transmission of malaria.

Factors which influenced the malaria rates presented in this paper included amount of malaria resident in the troops, the status of quinacrine suppressive therapy, the extent of the local control problems and the promptness and effectiveness of mosquito control measures. These are discussed in order.

Efate, Espiritu Santo, Guadalcanal, and Tulagi-Florida were in large part originally occupied by previously uninfected troops. In late 1942 and in the first half of 1943, a very great number of troops on Guadalcanal became infected with malaria. This included the 1st and 2nd Marine Divisions, the Americal and 25th Divisions, the 147th Infantry, to a lesser extent the 43rd Division and many personnel of smaller units, Army, Navy and Marine. The 37th Division, on Guadalcanal at this time, did not become heavily seeded, and never showed a high incidence of malaria. These heavily seeded units constituted a large proportion of the personnel who were to occupy bases established at a later date, and markedly influenced the malaria rates on those bases. The presence of heavily seeded troops on bases occupied later in the campaign often made it difficult to determine the degree of local malaria transmission. Occasional organizations arriving directly from non-malarious bases or from the United States furnished a valuable index.

The history of suppression is given in Paper I, and changes in parasite species partition in Paper IV. The dose of 0.4 gram quinacrine per week which was generally given until January 1944 was usually poorly supervised. This dose was insufficient to protect the majority of individuals and even in the conscientious individual often failed to suppress clinical malaria. The weekly suppressive dose was increased to 0.6 gram per week in January 1944 and malaria rates were reduced to negligible levels even in heavily seeded organizations, provided supervision was adequate. This must be considered in evaluating the data. The complete discontinuation of quinacrine on many bases and the gradual reduction in the number of troops taking this drug on all bases was associated with a continued fall in malaria rates and was evidence of the effectiveness of mosquito control measures. It cannot be emphasized too strongly that the control of malaria in the South Pacific resulted from the reduction of anopheline mosquito populations because of field control measures on all malarious bases.

Local control problems and the prompt institution of effective anti-mosquito measures were of great importance in influencing the malaria transmission picture. Guadalcanal, with about 110 square miles included within the perimeter, presented malaria control problems of the greatest magnitude because of exten-

sive tracts of low lying marshy ground and numerous land-locked lagoons. Certain of the smaller bases, such as Tulagi, Treasury, Green and Emiru never presented serious technical difficulties. Efate, Santos, the Russell Islands, New Georgia and Bougainville presented problems of intermediate difficulty. Control work other than hand larviciding was delayed for many months after occupation on all of the bases occupied in the first 1½ years of the campaign. Indeed, larviciding was not started on Efate or on Guadalcanal until a malaria epidemic was well under way. Malaria control personnel accompanied or landed shortly after the occupying forces and started control work immediately on the Russell Islands and on all subsequently occupied bases. Even on these bases, however, it was 6 months or more after occupation before semi-permanent control projects requiring heavy equipment were initiated.

It is clear that early insect control measures undertaken by an organization such as that developed in the South Pacific, which is adequately supported and supplied, can prevent malaria and other insect-borne diseases from jeopardizing the success of military campaigns in the tropics. Field control measures directed against disease bearing insects are the basis of any sound program. Suppressive drug therapy and other individual protective action are auxiliary measures of varying value.

F. SUMMARY

Malaria rates of all forces on the major bases in the South Pacific Campaign are presented with brief histories of each base. Similar data are presented for selected army units which proceeded from base to base. A brief account of the dengue epidemics on New Caledonia and Espiritu Santo is given.

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MALARIA AND OTHER INSECT-BORNE DISEASES IN THE SOUTH PACIFIC CAMPAIGN

1942-1945

III. ENTOMOLOGY

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The island bases constituting the South Pacific area have been enumerated and discussed elsewhere.¹ The entomological report here presented applies to the 11 malarious bases only unless other islands are specifically indicated, and most of the information given is drawn from observations made on Efate, Espiritu Santo, Guadalcanal, and Bougainville. Whenever possible reference is made to published data used in connection with the preparation of this paper, but we have also drawn freely from unpublished reports prepared by the numerous entomologists who at various times contributed to our knowledge of problems of medical entomology arising from our military occupation of these tropical bases.

Little detailed information regarding the insect fauna of these islands is available, and it is not within the scope of this paper to attempt generalizations as to the origin or affinities of the fauna. However, it is interesting to note that while only 12 species of mosquitoes are known from New Caledonia and 18 from Espiritu Santo, the largest of the New Hebrides group, there are now approximately 70 species known to occur on Guadalcanal in the Solomons group. Prior to occupation by armed forces but approximately 30 species of mosquitoes were known from the entire Solomons. Bougainville, though to date less completely explored, may be expected to have an even richer fauna than does Guadalcanal.

The population of all these malarious bases is predominately Melanesian, excepting Efate and Espiritu Santo where a considerable element of Tonkinese (French Indo-Chinese) occurs. Whites are few. Sanitary conditions, particularly among the Melanesians, are primitive, and the thatched hut dwellings so characteristic of the South Pacific islands offer easy access to mosquitoes and flies (see fig. 1). In addition the Melanesians are confirmed travelers and frequently spend the night along beaches or trails between villages, at which time they undoubtedly serve as a source of infection to mosquitoes in the more isolated regions of the islands. It is small wonder then that malaria is hyperendemic throughout these islands.

Pigs and chickens are common, the former abundant in a wild or semiwild state as well as being domesticated. Horses and cattle, except in the vicinity of plantations, were few, and most of the latter disappeared from the occupied zones

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¹ Malaria and Other Insect-borne Diseases in the South Pacific Campaign, 1942-45. Paper No. 1 of this series: I. General Aspects and Control Measures.

of the Solomons soon after the arrival of large numbers of troops. Dogs were not common and cats were practically unknown until military occupation permitted their increase. One arboreal marsupial, *cuscus* is present and rats and bats are common. There is a great variety of birds, and at least two species of large, tree-climbing lizards in the Solomons. Snakes are not generally common but on Bougainville several species occur in conspicuous populations. Crocodiles occur in the coastal lagoons and small sluggish streams.



FIG. 1. VIEW OF NATIVE VILLAGE, GUADALCANAL, SHOWING TYPICAL THATCH SHELTER CONSTRUCTION

A. TOPOGRAPHY AND CLIMATE

1. Physical features

The general physical features and origins of the islands have been discussed in paper no. 1. Here it is proposed to point out the more important physical features of the occupied zones with particular attention to their bearing on entomological problems encountered.

Efate (New Hebrides).—A relatively small occupied area with few permanent streams and no large rivers. The coastal sections are underlaid with coral and with hills extending to the sea. Coconut, coffee, and cacao plantations are common.

Espiritu Santo (New Hebrides).—Occupied area approximately 40 square miles in the southeastern portion of the island, consisting of a long, narrow, coastal strip extending from Turtle Bay on the north to the Renee River area on the south. Numerous large streams and rivers, some of them underground and

emerging from coral craters near the coast. The larger rivers subject to considerable variation in flow, often becoming swollen and rapid in rainy seasons. Upon receding, these rivers leave numerous pools behind sandbars and in catchments along their courses. The banks of smaller streams usually with heavy jungle growth which often overhangs into the water in such a manner as to impede flow or form lodgment for aquatic growth. Coastal sections underlaid with coral and with hills extending to or near the sea. South of the Renee River numerous ponds occur. Coconut, coffee, and cacao plantations are common. Soil sandy near the coast but quickly giving way to a fine textured, relatively impervious clay. Swamps few in number and these usually limited in extent. Areas not cultivated are covered by a dense rain forest. Numerous smaller islands off shore, some of which were occupied by troops. On some of these small islands *Anopheles* were never found during the course of military occupation and malaria was not a problem.

Guadalcanal (Solomon Islands).—The occupied area consisted of approximately 110 square miles along the northwest coast and included approximately 45 miles of coast line. This area, east of the Lunga River, consisted of a broad alluvial plain varying in width from one to 10 miles, transected at intervals of approximately 3 miles by major drainage courses arising in the high elevations to the south. Between these rivers (Lunga, Teneru, Nalimbu, Metapona, and Balasuna) are numerous sluggish, meandering streams representing old river beds in the process of being resedimented. Most drainage courses in this area, both rivers and small streams, are bordered by heavy jungle; between these jungle areas are broad open grasslands extending back into the foothills. Beaches in this section are composed of dark volcanic sand. Soil, except for beach strip, consisting of fine black silt, high in organic content, overlaying a yellow clay subsoil. The topsoil, when undisturbed, was capable of absorbing an enormous amount of water, but once rutted and packed by vehicular traffic became highly impervious. West of the Lunga River valley the hills approach or reach the sea, the permanent streams are fewer and smaller, and many of the secondary drainage courses are dry except during periods of abundant rain. The soil is underlaid with coral and for the most part sandy and coarser than in the eastern portion of the occupied zone. All small streams are periodically blocked at the mouth by a high, pounding surf, forming lagoons of fresh or brackish water. These lagoons sometimes remained blocked for a sufficient length of time to spread into the adjacent low areas and form coastal fresh water swamps. Adjacent to the foothills are numerous fresh water swamps, some jungle covered, others with a heavy covering of emergent aquatic or semiaquatic vegetation. Oxbows are common along the large streams in the broad alluvial plain east of the Lunga. Coconut plantations are common west of the Teneru River.

Tulagi-Florida Group (Solomon Islands).—The occupied area was small. The numerous small islands are without major drainage courses and with little permanent water. Hills extend to the sea and the coastal section is underlaid with coral. Coconut plantations are common. All entomological work was by Navy personnel.

Russell Islands.—The occupied area was small. The small, low islands are without major drainage courses and have little permanent water. Hills extend to the sea and the coastal section is underlaid with coral. Coconut plantations are common. Entomological work was entirely by Navy personnel.

New Georgia (Solomon Islands).—Approximately 20 square miles of occupied area in which the thin layer of soil is underlaid with coral. There are no large streams and few small ones. A few swamps were present in or adjacent to the occupied area but these were not utilized by *Anopheles farauti* as they were deeply shaded.

Bougainville (Solomon Islands).—The Bougainville perimeter was first stabilized to include an area of 30 square miles and later expanded to 50 square miles. The soil differs from that on other bases in that it is essentially volcanic sand for some miles inland. The terrain consists largely of a series of sandy terraces transected by a few streams and rivers, some of which were clogged by the debris of centuries with the result that each sandy terrace contained a series of swamps. A high water table, which quickly filled "fox holes" and shell or bomb craters, characterized many areas. The eastern portion of the occupied area consists of foothills, often steep, which lie at the base of high mountains and an active volcano. As on Guadalcanal, streams periodically blocked at the mouth by sand bars thrown up by a high, pounding surf, forming lagoons and coastal swamps. No extensive plantation areas. Jungle with dense undergrowth. Subsoil drainage good.

Green Island.—Coral atolls with only a thin layer of soil, a few coconut plantations, and some jungle. There was a limited insect fauna and no major control problems. Entomological work on Green Island was entirely by Navy personnel.

Treasury and Emir Islands.—Primarily coral with a thin topsoil, without large streams and with but a few swamps which were easily drained to the sea. The insect fauna limited and not involving major control problems.

Vella Lavella.—The occupied area was a narrow coastal strip where the soil was underlain with coral. Hills came almost to the sea. The area was transected at frequent intervals by small streams and contained a few swamps which were easily drained to the sea once adequate equipment was available.

2. Climatological

Available climatological data are given in paper no. 1. On Guadalcanal rainfall for 1944 totaled 87.74 inches at the Henderson Field weather station and 81.69 inches at Carney Field. Complete data are not available for Doma Cove at the western end of the occupied zone but the total rainfall was approximately that recorded for Carney Field. February was the month of heaviest precipitation at all stations, and more than 8 inches of rainfall was recorded at all stations on each of the first 4 months of the year. The distinction between the wet and dry seasons was not marked, and at Henderson Field the rainfall was above the recorded 25-year average for each month except January, November, and December. The daily temperature range was approximately 20°F., the maximum sel-

dom being above 92°F., or the minimum seldom below 69°F. The average relative humidity was seldom below 80 for any month.

These data for Guadalcanal are fairly representative of all the major bases. However, the distinction between wet and dry seasons was more pronounced in the New Hebrides and less evident on Bougainville. The total rainfall on Bougainville was considerably higher than on Guadalcanal. It is emphasized that the "dry season" and "wet season" are relative terms. During most months of the year there was sufficient rainfall to maintain surface water in all except the smallest catchments, and the abundant rainfall and relatively high temperatures and humidities provided conditions ideal for the development of large mosquito populations.

Seasonal changes in the entomological problems, particularly with respect to mosquitoes, were influenced largely by rainfall. Following the onset of heavy rains at the beginning of the wet season, floodwater *Aedes* would become abundant and at times troublesome. Flooding of normal breeding areas of such forms as *Culex* and *Anopheles*, so that the available water surface was increased many times, served to disperse the current larval population and this gave the impression that there was a cessation of breeding. To some extent this was probably true, since torrential rains caused flushing of some surface pools and larval development, in exposed situations, was probably inhibited by the constant or frequent agitation of the water surface and the lowering of the water temperature. Periods of heavy rainfall, with the consequent flushing of drainage courses, resulted in temporary elimination of breeding in most rivers and streams.

On most bases, the peak of *Anopheles* population curves usually occurred soon after the end of the season of heavy rains. A number of factors apparently contributed to this situation. The stabilization of abundant surface water, increased sunlight and its resultant effect on development of plant life, and higher water temperatures, combined to make conditions ideal for larval development. Larvicidal crews were sometimes temporarily overwhelmed with work and unable to reach the more inaccessible breeding areas. This situation could usually be corrected in a short time, the speed with which it could be accomplished being dependent upon the progressive drying up of pools and the gradual improvement in larvicidal coverage.

The advent of the dry season usually meant an abrupt dropping off of the abundant populations of flood-water *Aedes* except in coastal jungle areas where diminished populations frequently persisted throughout the year. As sunlit surface pools decreased in size they frequently became unsuitable for *Anopheles* development and *Culex annulirostris* would be the only species of mosquito to utilize such places. On bases characterized by a fairly evenly distributed rainfall such as Bougainville, *Anopheles* and other mosquitoes were generally abundant at all times unless control measures were instituted. On Bougainville increase of *Anopheles* populations was most rapid during the frequent prolonged periods of light afternoon showers which served to keep all water catchments filled without subjecting them to flushing action.

B. ENTOMOLOGICAL PROBLEMS ENCOUNTERED

1. *Malaria Vectors*

Anopheles (Myzomyia) farauti Laveran was by far the most important and probably the only important malaria vector in the South Pacific area, at least from a military standpoint. Other species of *Anopheles* encountered, namely *punctulatus* (S. & S.), *lungae* B. & S., *koliensis* Owen, *solomonis* B., K. & R., and *nataliae* Belkin, while on occasion abundant in restricted habitats were seldom present in sufficient numbers to be considered major factors in malaria transmission. Of these species, *koliensis* is strongly anthropophilic and thus potentially an important vector, *punctulatus* feeds freely on man in captivity but apparently only rarely in nature, while the remaining species are apparently largely dependent upon native wild hosts for blood meals. Details of available information concerning these species may be obtained from published articles.²

In addition to being an exceedingly efficient vector, *A. farauti* exhibits adaptability to a great range of habitats for larval development. Although the highest concentrations of larvae were usually found in sunny, slightly brackish lagoons in association with emergent vegetation or flotage, the species effectively utilizes almost every conceivable type of aquatic habitat with the exception of artificial containers, tree holes, open moving water and highly saline water. In the course of investigations in the South Pacific Area, larvae were found in temporary water catchments of many kinds, in streams, rivers, ponds, seepage areas, open wells, and both fresh and tidal swamps and lagoons. Prior to occupation of an area by troops, larval populations appeared to be largely restricted to lagoons, streams, and rivers. Following occupation and the consequent creation of innumerable road ruts, "fox holes," borrow pits, and road ditches, *farauti* quickly utilized all these places and in the absence of control operations developed enormous populations. *Farauti* does not commonly occur in heavily shaded areas, and its occurrence in swamps is usually restricted to the more open sections.

Both *koliensis* and *punctulatus* are found in typical *farauti* habitats in the larval stages, especially during the wet season, although *punctulatus* apparently disappears from the coastal section of Guadalcanal during the dry season. *Lungae* occurs in coastal swamps and seepage area, and shows a decided preference for shaded habitats. *Solomonis* and *nataliae* both occur in the low foothills section along the northwest coast of Guadalcanal, the former being collected from pot

² Belkin, John N., *Anopheles nataliae*, a new species from Guadalcanal. Jour. Parasitol., 31(5): 315-318, 1945.

Belkin, John N., Knight, Kenneth L., and Rozeboom, Lloyd E., Anopheline mosquitoes of the Solomon Islands and the New Hebrides. Jour. Parasitol., 31(4): 244-265, 1945.

Belkin, John N., and Schlosser, Ralph J., A new species of *Anopheles* from the Solomon Islands. Wash. Acad. Sci. Jour., 34(8): 268-273, 1944.

Daggy, Richard H., The biology and seasonal cycle of *Anopheles farauti* on Espiritu Santo, New Hebrides. Ent. Soc. Amer. Ann., 38: 1-13, 1945.

Owen, William B., A new anopheline from the Solomon Islands with notes on its biology. Jour. Parasitol., 31(4): 236-240, 1945.

Perry, William J., Keys to the larval and adult mosquitoes of Espiritu Santo (New Hebrides) with notes on their bionomics. Pan-Pacific Ent., 22: 9-18, 1946.

holes in a coral stream bed and from seepage areas, usually in clear water, deeply shaded, while the latter was taken from densely shaded, clear running water in seepage or spring areas. *Koliensis* has been found only in the alluvial plain area east of the Nalimbu River on Guadalcanal.

Females of *farauti* and other species of *Anopheles* encountered are primarily nocturnal in their blood-feeding habits, although daytime biting by *farauti* under favorable conditions was not uncommon. Following blood meals the females frequently remain in darkened portions of native huts or closed tents throughout the following day but in open tents such as were used by troops there was little evidence that the mosquitoes lingered after feeding. The diurnal resting places of *farauti* apparently consist of any cool, moist, shaded places. With the exception of blooded females no large concentrations of adults in diurnal resting places were encountered.

There is considerable evidence to indicate the existence of at least two races or physiological strains of *farauti* on Guadalcanal. It was repeatedly observed that adult females reared in large numbers from larvae collected outside the zone of normal human habitation would not feed readily on humans in captivity. Attempts to collect adults at night in such areas, even where larval populations were extremely high, invariably resulted in almost complete failure. On the other hand insectary colonies established from eggs obtained from blooded females collected in native huts, or from larvae collected in the vicinity of human habitations, usually produced females that fed readily in captivity. While inconclusive, such evidence strongly indicates that these "wild" populations are maintained by blood meals taken from birds, pigs, or other wild animals. Host preference tests conducted on Efate indicated that *farauti* would feed readily on cattle, horses, goats, pigs, dogs, and chicken, and that both cattle and horses are preferred to man for the blood meal.

These remarks are not intended to imply that these physiological strains, if they do exist, are fixed in their food habits. On the contrary it is believed that these "wild" strains could and did adapt themselves readily to humans whenever the opportunity offered. For this reason, in recommending control operations, no distinction was made between presumed "domestic" strains and those believed to be "wild."

In considering control operations in the South Pacific Area, it should be remembered that they were initiated and developed on most bases before DDT with its enormous labor-saving potentialities became available or even known. Two alternatives were thus available: either an extensive, never-ending, and sometimes an all but impossible larvicidal program, or a planned elimination of breeding places. In actual practice both alternatives were of necessity followed. A larvicidal program was begun at the earliest possible time, and as equipment and labor became available semipermanent control projects were undertaken. On most bases planned elimination of breeding places was never sufficiently effective to obviate the necessity of a thorough larvicidal program. Elimination of breeding places was of great importance however, since it served to bring the total

water surface within the capacities of available larvicidal crews and at the same time contributed greatly to the overall reduction of mosquito populations. It was the entomological survey group's responsibility to determine the projects to be undertaken and to participate in determination of work priorities.

Since larval populations in permanent water courses are usually associated with vegetation, a program of stream cleaning was recommended. This accomplished the desired result in three ways: it eliminated protective vegetation, it made the remaining breeding foci accessible to larvicidal crews, and it insured swifter flow of water with consequent flushing action in the streams following



FIG. 2. A SLUGGISH STREAM IN NEED OF RE-CLEANING

In streams with sufficient fall flushing dams would effectively prevent formation of algal mats and to some extent the regrowth of marginal vegetation. Photo No. 44-4796, 161 Photographic Company.

rains. Frequent reeleaning was necessary (see fig. 2). In stream courses with sufficient fall, flushing dams were recommended. These proved effective.

Control of *Anopheles* in lagoons depended to a considerable extent upon the elimination of vegetation and flottage. This could usually be accomplished by periodic opening of the lagoon to the sea to permit drainage or tidal action and salinification. Where barrel-type flumes were installed and properly maintained they proved to be one of the most effective and economical of the control methods. It was first believed that the increased salt content of the water was responsible for cessation of *farauti* breeding, but this factor alone was found not responsible except as it contributed to the elimination or change of vegetation. Careful

tests of the saline content of the water in many lagoons so treated showed that in no instance was the salinity of the water above the known tolerance for *farauti* larval development. In addition to the flushing action created by the installation of flumes there was also a great reduction in water surface.

By far the greatest source of widespread breeding of *A. farauti* was in the innumerable man-made catchments, in which at times, it is estimated that 90 per cent of the breeding occurred. The most important of these were ruts, improperly constructed ditches, and abandoned "fox holes." Coconut, coffee, and cacao groves, with their excellent camouflage, were widely used for supply dumps



FIG 3. ANOPHELINE BREEDING SITES ON BOUGAINVILLE CREATED BY AN ARTILLERY BARRAGE

Photograph by Major John Weir

of all kinds with the result that miles of ruts were cut as vehicles traveled up and down row after row of trees. Thinned jungle areas used for supply dumps, and the omnipresent logging operations created extensive rutted areas in a similar manner. On Guadalcanal the extensive grasslands which permitted indiscriminate movement of vehicular traffic proved to be a serious and long continuing problem. Eventual elimination of rutted areas was accomplished by thorough disking, associated with proper ditching and road construction.

Shelling and bombing in combat areas proved to be only minor factors in the creation of *Anopheles* breeding sites. On Bougainville, however, a concentrated air and artillery barrage of enemy positions later occupied by our troops presented serious local problems which were quickly abated by filling operations (see fig. 3).

2. Dengue Vectors

But one certain vector of dengue fever, *Aedes (Stegomyia) aegypti* (L.), the yellow fever mosquito, occurs commonly in the South Pacific area. In habits primarily a domestic species, it was apparently responsible for severe outbreaks of dengue on New Caledonia, Efate, Espiritu Santo, and Tulagi-Florida in 1943 and for a mild outbreak on New Caledonia in 1944. Until November 1943, when a localized infestation of *aegypti* was found on Guadalcanal, the species was not known to occur in the occupied portion of that base. Critical survey work to determine the limits of this infestation, followed by a diligent clean-up program, resulted in the elimination of this infestation by midyear in 1944. There is no reason to believe that any cases of dengue resulted from this temporary infestation of *aegypti* on Guadalcanal.

There is considerable reason to believe that *Aedes* of the *scutellaris* complex may at times be involved in the transmission of dengue. Daggy³ has reported on a dengue epidemic on Espiritu Santo in which a member of the *scutellaris* complex, *hebrideus* Edw., appeared to be an important factor. There is need for considerable critical work in order properly to evaluate the role of various species of *Aedes* as vectors of dengue fever.

Aegypti is commonly found only in the immediate vicinity of human habitation, where it breeds in almost any kind of artificial container. In the New Hebrides and Guadalcanal, under conditions of early military occupation, it found abundant favorable conditions for development in truck tires piled in supply and salvage dumps. In New Caledonia, where there is a concentrated civilian population, the chief source of breeding was in water containers around dwellings and in the numerous flower vases in cemeteries. Although not so completely domestic, the members of the *scutellaris* complex breed in most situations that are suitable for *aegypti* but also utilize a great many types of situations such as tree holes, coconut husks, and the like. The thoroughness of the control program, primarily one of survey inspection and elimination of breeding places, was directly responsible for reducing or preventing dengue outbreaks after the severe epidemics of 1943.

3. Filaria Vectors

Filariasis is common among the Melanesians in the South Pacific, but was not a problem of military importance except in the Polynesian islands, where the vector is culicine, *Aedes (Stegomyia) pseudoscutellaris* (Theo.). A summary of the relation of the *scutellaris* group of *Aedes* to filariasis is given by Farner and Bohart.⁴ Little was known concerning the vector of filariasis in the New Hebrides and Solomons at the time of occupation of those bases, but subsequent investigations indicate that the disease was transmitted largely by *Anopheles*, particularly *farauti*, although *koliensis* appears abundantly capable as a vector. Although a number of species of mosquitoes are capable of picking up the micro-

³ Daggy, Richard H., War Med., 5: 292, 1944.

⁴ Farner, Donald S., and Bohart, Richard M., Naval Med. Bul., 44(1): 51, 1945.

filaria, few harbor the worms to the infective stage. Available data in unpublished reports are based upon dissection of collected material and upon dissection of specimens fed on known infected Melanesians.

Because the vectors of filariasis were those of primary concern from the standpoint of malaria transmission, in the Solomons and New Hebrides the control of filariasis was solved with the reduction of *Anopheles* brought about by malaria control activities.

4. Pest Mosquitoes

Aside from their importance as vectors of disease, mosquitoes were frequently of considerable importance in restricted areas because of their annoyance. This applied to both combat and rear area troops. No measure or even accurate estimate of the importance of this annoyance in the prosecution of the war is possible, but it must be recognized as considerable purely from the standpoint of lessened efficiency. In addition, it was probably at times a contributory factor in a general lowering of morale, psychoneurosis, and even loss by enemy action. On the other hand, the presence of pest mosquitoes, whenever they occurred in numbers, probably greatly increased the extent of observance of individual protective measures and was therefore of some influence in reduction of malaria incidence. Among the species of mosquitoes that assumed importance purely from a pest standpoint are:

Aedes (Ochlerotatus) vigilax Skuse.—This species, breeding in the salt and brackish marshes of New Caledonia, was blown or drifted in countless numbers into the inland valleys of that base. As a result, sections that would have been ideal for hospital sites and rest areas were at times thoroughly plagued by a fierce and persistent daytime biter. Under such circumstances outdoor activities, particularly swimming, became an ordeal rather than a relaxation. To some extent these populations could be reduced by airplane spraying with DDT, but observations following such area treatment indicate that the effect was extremely transitory and that adult populations were back to normal after about one week. Claims of nearly complete elimination of the adult population, and a lag of two to three weeks in reinfestation of an area are apparently without basis.

Aedes (Aedes) funereus (Theob.), *A. (A.) ornatus* (Theob.), *A. (Aedimorphus) vexans* Meigen, and *A. imprimens* (Walk.), all floodwater species, were at times abundant and extremely annoying in jungle or coconut-grove sections on the bases where they occurred. They are fierce and persistent daytime biters, but like most *Aedes* have a short flight range and were not troublesome far from breeding areas. Most instances of annoyance in camp areas could be traced to local water catchments. Except for the deeper jungle areas these species largely disappeared during the dry season.

Aedes (Stegomyia) hebrideus Edw. and *A. quasiscutellaris* F. & B. breed in tree holes, coconut shells, tin cans, and artificial water catchments of all kinds. They are apt to be the most abundant daytime biters and are very persistent and annoying. Normal clean-up measures such as are directed against *aegypti* will usually suffice to reduce the population to a negligible point.

Culex annulirostris Skuse.—This is by far the commonest mosquito in the New Hebrides and Solomon Islands where it breeds in enormous numbers in all kinds of natural water catchments. It will utilize pools long after they are unsuitable for *Anopheles* development. It is primarily a night biter and was consistently taken in night population samples when other species of mosquitoes were extremely scarce. It does not commonly occur in deep jungle areas, seeming to prefer the more open coconut groves and thinned jungle areas that were so extensively used for bivouac sites.

5. Flies

With relatively few exceptions flies were of little consequence in the South Pacific area, but when abundant, or permitted to become so, were believed to be important in connection with dysentery outbreaks, in addition to being extremely annoying. On Efate and Espiritu Santo, early in 1943, two species, *Musca sorbens* Wd. and an undetermined species, were present in great numbers, and clinical evidence suggested that these flies were in large part responsible for outbreaks of bacillary dysentery. In addition to their presence in mess halls and latrines these flies were constantly present during daylight hours outdoors. They were extremely persistent in their attempts to feed on any open sore or wound, and if given the slightest opportunity would fly into the mouth, nostrils, eyes, or ears. Normal sanitary measures and the removal of cattle from adjacent groves where they were customarily permitted to graze usually sufficed effectively to curtail these *Musca* populations.

In the Solomons, and to some extent the New Hebrides, *Chrysomya megacephala* (F.) was extremely abundant in and around garbage pits, trash dumps, and latrines. It did not habitually frequent mess halls. Normal sanitary measures would control this species.

On Guadalcanal, *Hermelia illucens* (L.) was sufficiently common in latrines to earn the name "latrine fly." It was chiefly of annoyance and so far as known has no importance in connection with disease transmission.

Sand flies, *Acanthoconops albiventris* (Meij.), were abundant and troublesome on some beach areas on Guadalcanal. They were restricted to sandy beaches and usually to sections within a quarter mile of a beach lagoon. Investigations indicate that optimum conditions for larval development occur only in a relatively narrow strip of beach near the high tide line, and the greatest concentrations of larvae appear to be just inside the mouth of lagoons where the sandy beach grades into the finer silt and muck. No larvae were found, their occurrence being indicated by adult emergences. *Acanthoconops* bites freely during the daytime. The bite is not painful but causes rather severe reactions in some individuals. The species was chiefly of concern as an annoyance in recreation areas and is not known to transmit any disease on Guadalcanal. Temporary reductions of adult sand-fly populations were obtained by area treatment with DDT oil solution and with DDT thermal generator applications. Sand flies were also abundant and troublesome in troop recreation areas along beaches, and in combat beach areas on Bougainville.

6. Other Arthropods of Importance

Scrub typhus, transmitted by trombiculid mites (chigger mites), was encountered in the South Pacific on Espiritu Santo, Munda (New Georgia group), and Bougainville, but only on the last-named base was there evidence of any considerable reservoir of the disease. Individual protective measures, particularly avoidance of likely mite habitats and use of dimethyl phthalate on skin and clothing appeared to offer the best insurance against contracting the disease. Information as to the efficacy of cutting and burning vegetation thought to harbor the mites and their larval hosts is not conclusive.

Ants (species unknown) were abundant everywhere in the New Hebrides and Solomons. They were of importance only as a nuisance, being attracted to any available sweet or oily delicacies such as candy, cookies, and peanuts. Occasionally they established nests in clothing or other impedimenta left undisturbed for a few days. DDT, applied to screens and around the floor of quarters was highly effective against ants and virtually eliminated them as pests.

On Bougainville, and to a lesser extent on some other bases, an unusually large centipede common throughout the occupied area was the cause of much annoyance. The bite was exceedingly painful and was often followed by reactions so severe as to require hospitalization.

C. ORGANIZATION OF SURVEY ACTIVITIES

A personnel organization for entomological survey activities that proved adequate for the average area assignment follows:

DESIGNATION	NUMBER	DUTIES
Entomologist	1	Officer (Army SnC or Navy H-V(S)) in charge
Senior NCO	1	Direct supervision of field and laboratory work
Laboratory and insectary men	2-3	Map making, record keeping, care and identification of material brought to or reared in insectary
Field men	5-8	Responsible for field surveys and reports of field conditions

The entomologist and his crew were responsible for, (a) surveys to determine the incidence, distribution, and biology of arthropods of medical importance, (b) recommendations as to the areas requiring control operations, the relative importance of these areas, and their proper treatment, (c) routine inspection surveys to determine the effectiveness of control measures, (d) maintenance of appropriate maps and records of insect populations and their fluctuations in response to control activities or other factors, (e) special investigations of immediate importance to control operations or to the health and welfare of troops, and (f) participation in the general training program.

It is emphasized that the personnel organization, and the responsibilities of the survey group, were plastic arrangements in actual practice, subject to change from day to day as conditions and problems encountered in the different areas varied.

Although constantly alert for any problems in medical entomology, primary

attention was nearly always directed toward the major problem, malaria. Consequently, the entire survey organization was planned to furnish information on mosquito populations and their control. Other activities were of a secondary nature. The entomologist necessarily devoted a great deal of time to field work, especially during the early stages of occupation of an area. It was necessary for him to be familiar with the entire assigned territory in order to interpret survey data intelligently, to recognize the types of problems to be expected, and to anticipate laxity on the part of the field crews. Frequent discussion of problems with the survey crew was encouraged and with inexperienced personnel this often involved interviews twice daily.

Field work by the survey team was usually assigned on the basis of sub-areas of from three to five square miles per individual or team. If two or more individuals worked together as a team the senior in rank was made responsible; if subareas were assigned to individuals there was individual responsibility under the direction of a designated field supervisor who worked in any or all subareas as the need arose. On some bases the entire survey crew worked throughout all assigned territory. This system had the advantage of not concentrating the work of the less efficient survey men in a single subarea or subareas, and of giving all portions of the territory the benefit of the work and observations of the most efficient survey men.

Whatever arrangement of personnel assignment was used, the survey crew was expected to cover its territory once each checking period so that a complete and continuous record of mosquito populations was available. Both weekly and fortnightly checking periods were used at different times and on different bases. Survey personnel were expected to furnish information not only on insect populations but also on the status of control projects, the location of potential hazards such as unauthorized can and garbage dumps, on the occurrence of new problems, and to make recommendations for the correction of problems encountered. As such items became available survey personnel also carried small hand sprayers for larviciding small water bodies found to need treatment.

Survey men assigned to laboratory and insectary work were charged with the responsibility of rearing specimens, identification of specimens caught in night catches, preparation of record maps at the end of each checking period, maintaining records of adult catches, assisting in experimental work, and helping with the usual office and laboratory routine. In addition, they were expected to spend some time in the field, either with members of the field crew or in independent survey activities.

In addition to the survey unit organization discussed above, on some of the larger bases such as Espiritu Santo, Guadalcanal, and Bougainville there was need for central coordination of entomological activities. To accomplish this end there was designated for each of these bases an "island entomologist" whose duties were to plan, coordinate, and supervise the general survey program and to advise the island malariologist concerning the broader aspects of the entomological work. This was not a command position, and the extent to which the desired cooperation was actually attained was to a considerable degree dependent upon

the extent to which the program could be sold to the island malariologist and the participating entomologists. In some respects the results were good, but experience indicates a very definite need for supervision of entomological work by an entomologist in whom is vested authority as well as responsibility.

The very nature and multiplicity of duties assigned to the entomologist and his crew usually made it impossible for him to carry out adequate investigations of all problems encountered. Whenever possible, essential investigations were carried out in cooperation with research units such as Naval Medical Research Unit No. 2, which sent advance parties to the South Pacific area. On some bases, a cooperative program of investigational work was developed under the direction of the island entomologist. As they arose, special problems were assigned by the island entomologist. Such problems included methods of application of DDT, toxicity of DDT, tests of repellents, surveys of special territories, and special studies of insect biologies.

On Guadalcanal and elsewhere meetings of all district and division entomologists were held monthly, or more often if considered desirable. These meetings were initiated by the island entomologist primarily for the purpose of discussing and clarifying administrative procedure. Once this phase of the entomological work was established the meetings were continued in order to permit an interchange of ideas and a general discussion of practical problems encountered. These meetings did much to further the coordination of entomological activities and aided materially in the development of a comprehensive investigational program designed to supply information of direct benefit to control activities. In addition there were developed standardized methods of larval population sampling and uniform methods of reporting, so that reports from various districts could be readily consolidated and compared.

Occasionally entomologists or other survey personnel were charged with functions quite distinct from normal survey activities. Among these were: (a) Development of a mobile unit to service aircraft used in aerial application of DDT, and working out an operational procedure for same. (b) Scheduling and supervising airplane spraying of DDT, including the activities of the ground service unit, and (c) participation in mosquito control programs, either by being made directly responsible for the larviciding phases, or through periodic assignment of survey men to larvicidal crews.

D. SURVEY DUTIES AND PROCEDURE

1. Initial Surveys

Ideally, the initial survey of a territory followed a critical study of maps and aerial photographs of the area. However, in actual practice, at least in the early phases of the South Pacific campaign, this was seldom possible because of the inability to obtain satisfactory maps or photographs. The original survey work was thus to a considerable extent exploratory in nature and designed to give a general picture of the physical features of the terrain and the problems involved. As the survey progressed, and at least within a few days, it was expected to

furnish preliminary information on the following: (a) location, extent, and description of actual or potential mosquito breeding places. (b) Records of larval populations. (c) Records of current adult populations. (d) Location of native villages, or other reservoirs of infection of tropical diseases and (e) conditions considered actual or potential hazards from the standpoint of insect borne diseases.

Control operations, particularly mosquito larviciding, were begun concurrently with survey work under most conditions. The initial surveys, while admittedly sketchy and incomplete in character, nevertheless directed attention from the outset to the most important and urgent problems existing in the area under consideration. As survey work progressed it was translated into specific recommendations for a control program, usually drawn up in conjunction with the sanitary engineer assigned to the district. An example of such a set of control projects developed on Guadalcanal and prepared by the entomologist of the district concerned is quoted:

HEADQUARTERS

MALARIA & EPIDEMIC CONTROL

APO 709

9 February 1944

Subject: Malaria and Epidemic Control Projects in the Tengeru Area.

To: Malariologist, Tengeru Unit, APO #709.

1. Projects for which the Island Malaria Control Organization is directly responsible.

A. Heavy equipment or dynamite work.

1. Ditch area west of 716 Medical Sanitary Company in vicinity of (95.5-91.5) into Little Tengeru River with sufficient laterals to drain entire area. Work now in progress. Work estimate, dragline for 10 days.
2. Extend ditching of upper Ellis, Chappel, and Parks Creeks, with sufficient laterals, to drain area in vicinity of (100.0-90.5). Work estimate, dragline for 14 days.
3. Drain area in vicinity of (101.8-89.4) possibly by ditch to Nalimbu River along telephone line. Work estimate, dragline for 14 days.
4. Ditch low area at west end of Nalimbu River bridge into Nalimbu River. Work estimate, bulldozer, leaning wheel grader, and hand ditching crew for 1 day.
5. Drain oxbow at (104.0-94.0) into Nalimbu River. Work estimate, dragline and bulldozer for 4 days.
6. Drain area in vicinity of (104.0-97.5) into Herr Creek or Cranford Creek. Work estimate, dragline and bulldozer for 3 days.
7. Fill or drain into Yankee Creek numerous potholes along lower Yankee Creek in vicinity of (102.8-96.6). Work estimate, bulldozer, dynamite crew and hand filling and ditching crew for 7 days.
8. Channel old creek bed at (104.6-98.2) and connect with Knapp Creek. Work estimate, dynamite crew for 2 days.
9. Fill potholes along Scott Creek. Work estimate, bulldozer and/or hand filling crew for 4 days.
10. Drain grassland in vicinity of (102.5-92.5) into ditch of highway 26 *after* ditching high 26. Work estimate, bulldozer and pull grader and hand ditching crew for 7 days.
11. Drain swamps near old 46 NCB rifle range on Lunga River at (86.3-90.4). Work estimate, dragline and bulldozer for 10 days.

12. Drain area adjacent to Fighter I into upper Ilu River. Work estimate, dragline and bulldozer for 14 days.
 13. Drain area between 20th Sta. Hosp. and radio station, vicinity of (90.0-91.0) into Tenern River. Work estimate, dragline and bulldozer for 4 days.
 14. Ditch area southwest of Mica Engineers, vicinity of (88.5-95.5), and connect drainage of area with Aviator Creek. Work estimate, dragline or bulldozer and pull grader for 5 days.
 15. Drain area south of 21st Medical Supply in vicinity of (93.7-92.5) into Little Tenern River. Work estimate, dragline for 3 days.
 16. Clear logs and old bridge from Little Tenern River at (93.7-91.5) and from (93.5-91.2) to southern extremity of controlled area. Work estimate, caterpillar and crew for 14 days.
 17. Services of level crew prior to initiation of all drainage projects are assumed. Work estimates do not cover time required by this crew.
- B. Hand cleaning by native crews.
1. Tributary to Parks Creek at (101.2-92). Work estimate, 50 man days.
 2. Herr Creek, especially upper end. Work estimate, 150 man days.
 3. Slough paralleling road to 13th A.F. at (101.1-96.0). Work estimate, 250 man days.
 4. Oxbow at (101.0-91.0). Work estimate, 1000 man days.
 5. Scott Creek. Work estimate, 200 man days.
 6. Mouth of Hook Lagoon. Work estimate, 50 man days.
 7. Upper Ilu River at (89.5-93.1). Work estimate, 200 man days.
 8. Tributary to upper Ilu River at (91.0-93.7). Work estimate, 200 man days.
 9. Ditch adjacent to Lunga River and highway 26 at (83.6-93.6). Work estimate, 100 man days.
 10. Maintenance work on streams. Work estimate, standing crew of 50 natives.
- C. Elimination of road ruts, puddles, fox holes, etc. in areas outside of bivouac sites. Work of this type may involve use of disk harrow, bulldozer, or hand crew. Principal territories where such work is needed are:
1. Road ruts at edge of grassland from vicinity of sawmill at (99.2-89.2) to the east.
 2. Fox holes on each side of highway 26 near Nalimbu River, vicinity of (104.0-90.5) and (104.5-91.4).
 3. Road ruts in grassland in vicinity of upper Oman Creek.
 4. Road ruts and small irregularities in the area west of 716 Medical Sanitary Company now being ditched.
 5. Fill well near Herr Creek at (104.0-98.0).
 6. Road ruts around Kiwi and Radar station in vicinity of (100.7-96.4).
 7. Road ruts between Hook Lagoon and Nalimbu River in vicinity of (105.5-99.5).
 8. Fill oxbow near mouth of Dodo River at (96.1-94.9).
 9. Road ruts throughout grasslands between Little Tenern River and White Creek.
 10. Road ruts in grassland south of 20th Station Hospital.
2. Projects of a public works nature falling for the most part under the general supervision of the Forward Area Engineer.
- A. Road ditches. The principal deficiencies in the present drainage system are those resulting from improper road construction and ditching. In order to insure ready run-off of surface water from the extensive grasslands in this area it is essential that road ditches, particularly those along the main roads, be cut to grade and the ditch system integrated with the natural drainage system. Further, it is essential that adequate and properly placed culverts be incorporated into this ditch system. Outstanding examples of deficiencies in road ditching are:
1. Highway 26 between Nalimbu River and Dodo River and between Ilu and Lunga Rivers.

2. Road north of highway 26 past 13th A.F. to the beach.
 3. Road to 37th Division combat range for $1\frac{1}{2}$ miles south of highway 26.
 4. Road from highway 26 to directional radio station at (100.8-91.1) and beyond to sawmill.
 5. Road from highway 26 past 20th Sta. Hosp. to radio station.
 6. Road between Henderson Field and Fighter I.
- B. Road Net. It is highly desirable that the agency responsible for the planning of a road net indicate where additional graded roads are needed so that such roads can be established and non-essential roads closed. Many bivouac areas are not now accessible by graded roads, hence vehicles drive through grasslands and continue to cut ruts which form a serious malaria hazard. It is impossible to eliminate road ruts so long as this condition is permitted to exist. This recommendation for the establishment of roads applies also to the road system in all ration, fuel ammunition, public works and salvage dumps.
- C. Airport drainage. There exist serious faults in the drainage system of Henderson Field and Fighter I. The Henderson Field drainage is particularly poor around the north and east sections of the field where numerous laterals are needed to connect with the main ditch already established. At Fighter I the revetments are nearly all surrounded by scooped out pockets which hold water constantly.
- D. Abandoned gravel pits along both the Nalimbu and Lunga Rivers are, at times, serious sources of *Anopheles* breeding. It is recommended that units working these pits be required to divert a channel of the river through each pit before the pit is abandoned.
3. Organizational work. It is recommended that all organizations in the area be urged to eliminate road ruts, puddles, and unused fox holes within and adjacent to their camp areas. Insofar as time permits Malaria Control will aid in this work, but such aid can be given only in projects of major importance. An example of such badly needed work is in the 27th NCB camp area and the adjacent Public Works Dump.
4. Dengue Control. In all salvage dumps, in the many unofficial and official trash dumps, and in the Ordnance Motor pool near 20th Sta. Hosp. many artificial containers are producing Culicine mosquitoes. While *Anopheles* may at times breed in such containers the primary hazard is from *Aedes aegypti*."

2. Maps and Mapping

Accurate maps are highly desirable, though not absolutely essential, in survey activities. Their value increases with the increase in extent and complexity of the area under scrutiny, and their greatest value is in expediting the translation of survey activities into actual control. Most military maps such as artillery fire control maps are unsatisfactory for detailed entomological survey work since they do not accurately show water courses and the scale is usually not adaptable to the needs of survey and control units. For this reason survey personnel frequently assumed the responsibility of preparing their own maps of the areas involved, and in some instances, as on Guadalcanal and Espiritu Santo, this activity was coordinated to produce a detailed map of the major portion of the occupied area (fig. 4).

Whenever possible aerial mosaics or photomaps were obtained. Aerial mosaics and contact prints of aerial photographs were found particularly helpful in making a critical analysis of a given territory. Mosaics were utilized a great deal in the preparation of preliminary maps of an area; these maps were then corrected and completed by ground inspection or available survey data. Large scale mosaics,

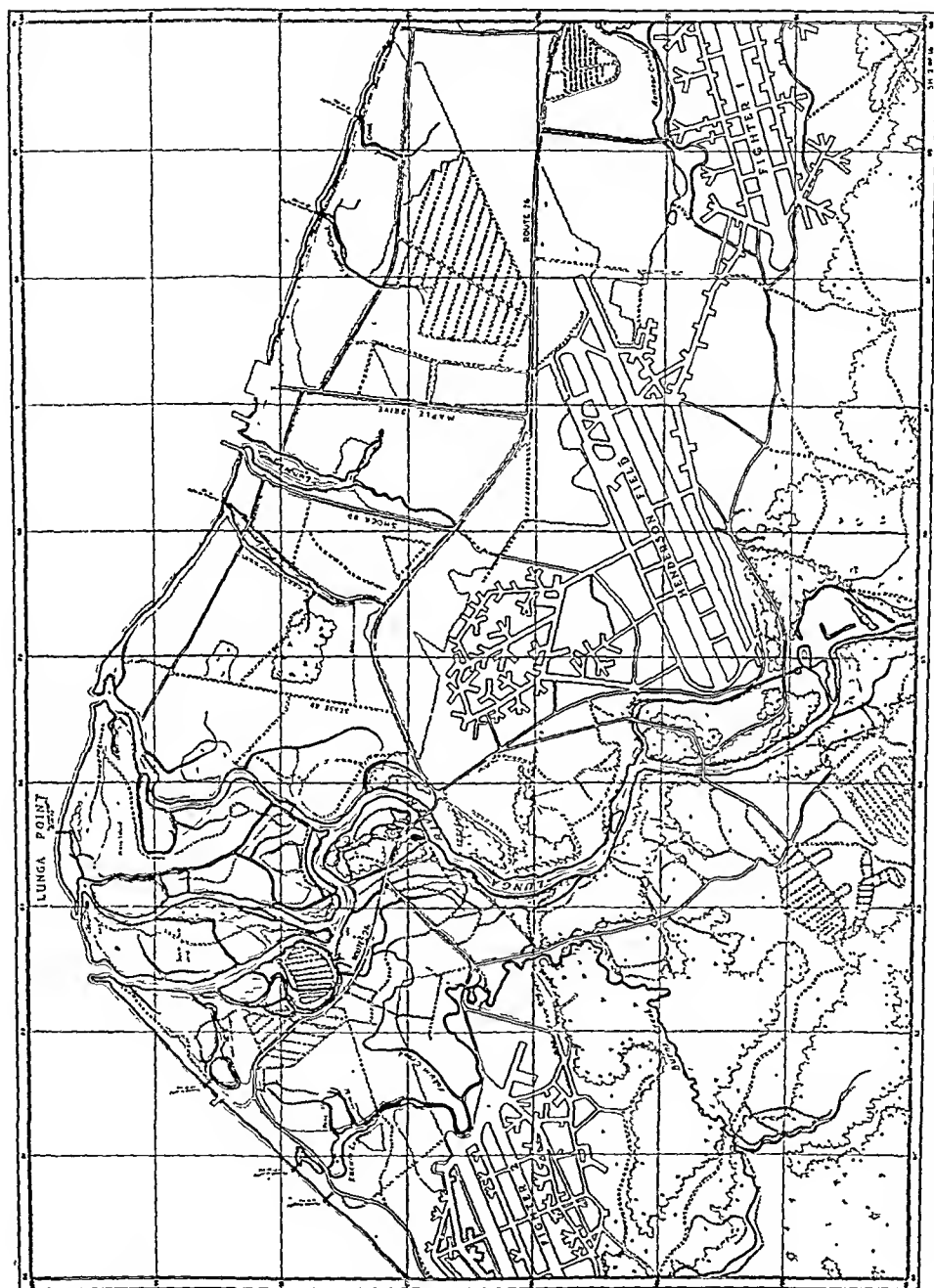


FIG. 1. SHEET OF 16 SHEETS OF MAP OF NORTH COAST OF GUADALCANAL PREPARED BY PERSONNEL OF THE 63RD NAVAL CONSTRUCTION BATTALION UNDER THE SUPERVISION OF THE ISLAND ENTOMOLOGIST, GUADALCANAL

This map, reproduced by lithographic process at scale 1:24000, was used for purposes of recording larval populations, engineering activities, and various other functions of the survey and control units.

preferable scale 1:5000, were used whenever possible for wall maps in the joint entomological and engineering laboratories (fig. 5). Covered with a transparent overlay of thin celluloid, these mosaics could be used for recording field data by use of colored grease crayons. In lieu of mosaics, large scale maps, reproduced by blue printing or direct printing on sensitized paper, were sometimes used for this purpose. Information recorded in this way gave a graphic presentation from day to day of insect populations, status of control operations and similar information.

Record size maps, scale approximately 1:30000, were used for reports of all kinds in summarizing field data. These were usually reproduced in quantity



FIG. 5. A PORTION OF AN AERIAL MOSAIC USED AS A WALL MAP ON GUADALCANAL, WITH A SHEET OF RECORD SIZE MAP FOR COMPARISON
Transparent overlay removed from mosaic for this photograph. Photo No. 44-6453, 161 Signal Photographic Company

by lithographic process, although often they were photographed or printed on sensitized paper. Reproduction work of this kind was usually done by photographic or engineering units at the request of the malaria and insect control organization. This mapping activity was not an authorized function of the malaria survey units, and the fact that it received the support of the various base commands is in itself evidence of its recognized value in the problems at hand.

3. Integration of Survey and Control Activities

Unless translated into control activities, survey data are of academic value only. Successful integration of survey and control activities depends upon constant liaison between the entomologist and sanitary engineer. Experience indi-

cates that this relation was best established and maintained when the personnel of the survey and control units lived as well as worked together, as was the usual practice in the South Pacific Area. The writers believe that the outstanding success of the malaria control program in the South Pacific was due more to the successful solution to this problem than to any other one factor. It is probable that even better coordination of activities would have resulted had the two functions been united under a single command.

Joint participation in the preparation of outlines of control projects mentioned above was one step in the process of integration of activities. This invariably involved discussion and examination of proposed ditching, cleaning, and filling projects by the entomologist and engineer with a consequent better appreciation of the purposes and difficulties in the work of each. It was endeavored to extend this understanding of problems to the enlisted men of the two commands by a similar, though necessarily less thorough program of joint participation in the various functions of the units.

Specific activities believed important in the integration of survey and control activities are, (a) use of large wall maps which show results of daily surveys for the information and guidance of control crews, (b) frequent assignment of survey men to accompany control crews and point out new breeding sites, and those that had been missed or inadequately treated, (c) use of flags in the field and on wall maps to mark exact locations of breeding sites, thus facilitating the work of the emergency oiling crews, (d) larviciding of small, isolated breeding sites by survey personnel, thus saving the larvicidal crew numerous trips into remote portions of the territory, (e) weekly conferences by survey and control personnel with the officers in charge, (f) use of prepared mimeographed slips giving location and exact information concerning faulty control activities, (g) investigations by survey personnel of problems having a direct bearing on control operations, (h) joint participation in recreational activities and general camp duties by men of the various units.

4. Routine Evaluation of Control Activities

As indicated in the preceding discussion, the survey unit was responsible for a constant and thorough check on the efficacy of control operations. The initial survey of a territory gradually evolved into a routine checking program as control operations became more extensive and complete. This program was basically an analysis of mosquito populations.

Even in areas of high mosquito populations it was impossible consistently to find adults of *A. farauti* in their daytime resting places, except for the blooded females that lingered in native huts and tightly closed tents. Native villages, including native labor camps in the latter phases of the campaign, were nearly always removed from troop bivouac areas, and mosquito population counts in those places were not representative of conditions in the occupied area. Night catches of mosquitoes seldom produced *farauti* in sufficient numbers to be significant, and as control operations progressed populations of all species of mosquitoes became so low as to render significant sampling difficult. For these reasons it

was felt desirable to rely upon larval sampling as a more sensitive index of changes in the population. Whenever adult populations were high, routine night catches were made to supplement larval population counts but as adult populations became meager night catches were discontinued. Light traps, though operated routinely for as much as six months on some bases, were never sufficiently productive to be considered accurate as a population sampling method.

In addition to an analysis of insect populations, the field survey crew was expected to report on work needed to correct potentially dangerous situations, even though actual breeding was not detected. This applied particularly to obstructions of streams and drainage ditching, flume maintenance, and occurrence of unauthorized trash and garbage dumps which might harbor *Aedes aegypti* or become foci of fly breeding. These checking functions covered areas controlled by all organizations, and involved liaison with the malaria and insect control personnel of line and service units of all kinds.

Officer personnel participated in regular inspection of organizations for observance of individual protective measures and general malaria discipline.

5. Records and Reports

In order to provide a basis for comparison of conditions in any one district with those in another, and of conditions within a given district from time to time, detailed records of all survey findings were kept. In addition, complete data on field collections and laboratory rearings of insect material were recorded and associated with material sent to scientific institutions in the States. Such records and material form a permanent record of the insect fauna encountered, and will be of inestimable value to war or peace time activity in tropical areas.

Although a variety of systems was used at different times and on different bases, all were designed to furnish the necessary pertinent data to determine the importance of the species involved. All specimens captured in routine night checks for adults were identified, listed by sex, and, in the case of females, recorded as to whether or not they had taken a blood meal. Sample larval collections were routinely brought in and identified or in many instances reared to the adult stage for subsequent identification. Findings of the field survey crews were recorded, for the information of the control personnel, as to whether they were *Anopheles* or other forms of mosquitoes. Where more than one species of *Anopheles* was found the species were differentiated in reports. Cognizance was normally taken of the presence of *Aedes aegypti*, especially on Guadalcanal where an attempt to wipe out the species was being made.

A common method of recording larval populations was to indicate the number of larvae found over the number of dips taken, thus 150/60, by writing these figures in the appropriate place on a transparent overlay to the wall map. *Anopheles* findings were recorded in red, other mosquito records in blue. The presence of late instars, indicating the necessity for immediate larviciding, was indicated by placing a "+" before the fraction. A locality flagged for attention by the emergency oiling crew was indicated by a red "F" on the overlay. The wall map was constantly consulted by all members of the control and survey

teams, as well as the malariologist. At the end of each checking period these insect population data were transcribed, by appropriately colored ink, to the record size maps for a permanent part of the reports on conditions in the territory.

On Espiritu Santo and Bougainville, survey areas were separated for record purposes into small subdivisions bounded by streams, hills, or other terrain features, or on the basis of territory occupied by specific troop units. In each of these area subdivisions the total number of sampling dips and the total number of *Anopheles* found were recorded during each inspection survey. On the basis of these statistics, translated into the number of *Anopheles* per 100 dips, a population index was computed. This population index proved to be of considerable practical value although on occasion it was somewhat misleading since it did not take into account changes in total water surface. By comparison with previous indices changes in the status of *Anopheles* breeding could be detected immediately in any part of the area surveyed and control emphasis could be directed toward specific localities where populations were becoming more abundant or not responding to treatment. Usually this population index would reflect increasing *Anopheles* densities in sufficient time to prevent accelerated malaria transmission rates by immediately instituting appropriate corrective action.

Responsibility for obtaining and recording pertinent climatological data, particularly rainfall, was undertaken as a survey function. Whenever possible these data were obtained from nearby meteorological stations maintained in connection with airfields; if such stations were not present records of rainfall were obtained by means of improvised gauges.

Monthly, or more often if deemed desirable, the entomologists prepared detailed reports of activities in their assigned districts. These reports covered such items as, (a) summary of survey activities and results, (b) climatological data and a discussion of the influence of weather conditions on insect populations and control operations, (c) reports on investigational projects, (d) participation in training programs, (e) recommendations for control and investigational work, (f) record maps and report forms giving details of insect population sampling.

6. Policy determination

At all times, but particularly in the initial phases of occupation of a new base or territory, the question of policy with respect to insect control methods arose. This was not alone the responsibility of the entomologist but was usually determined after exhaustive discussion of the problems with the malariologist and sanitary engineer. The entomologist, being responsible for recommendations for control procedure, was always vitally interested in this matter and usually in a position to furnish the bulk of the information pertinent to the question. Frequently, of course, policy would be determined by the type of equipment available for control work, in other cases by the decision as to whether control would be attempted largely by larviciding or by semi-permanent control projects. Experience showed that in stream cleaning it was usually unwise to use heavy equipment, see paper I, page 82. As a result it was recommended that all stream

cleaning be done by Melanesian laborers, or by troop hand labor. Decisions as to whether it was easier to fill or drain an area, ditching methods to be used, and so forth, were decided jointly with the engineer.

In general, under wartime conditions, decisions as to methods were made from the standpoint of speed of accomplishment and efficient utilization of available personnel and equipment rather than the dollars and cents cost of a project, since it was recognized that any other policy was potentially disastrous. The quickest control measures were ultimately the cheapest.

E. INVESTIGATIONAL PROJECTS

In addition to the more or less routine and standardized functions having a direct and immediate bearing on the problems at hand, whenever the need arose or conditions permitted the entomologist pursued investigational projects. While these may have a direct bearing on control work they are frequently of a basic character designed to broaden the general understanding of the problem. Details of our present knowledge of the mosquito fauna of the South Pacific Area were either acquired or confirmed by survey personnel working on insect-borne diseases during World War II. Attention of the entomologist was almost continually directed toward the following problems, (a) differentiation of the various species of mosquitoes occurring in the region, (b) host preferences, biting habits, night behavior, daytime resting places, and longevity of adult *Anopheles*, (c) factors determining or limiting suitability of water for mosquito breeding, (d) seasonal changes in the mosquito fauna and in breeding places of particular species, (e) length of the aquatic stages of *Anopheles* in nature, (f) effect of desiccation on *Anopheles* eggs, (g) survival of *Anopheles* larvae and pupae in moisture film on mud or vegetation, (h) susceptibility of larval and adult mosquitoes to certain larvicides and insecticides and its bearing on methods of mosquito control, (i) reaction of adult mosquitoes to repellents, (j) normal flight range of adult female *Anopheles*, and the existence of migrational or dispersal flights and (k) existence of races or physiological strains of *Anopheles farauti* and their importance in malaria transmission.

Other projects that periodically received considerable attention were: (a) determination of the local vectors of filariasis, (b) hosts of trombiculid mites, the vectors of scrub typhus, (c) incidence and species involved in human myiasis, (d) flight requirements for successful application of DDT sprays by airplanes, (e) droplet size analysis of airplane dispensed DDT, (f) suitability of various types of hand sprayers for use in application of larvicides, (g) methods of applying and value of applications of DDT to tentage, mosquito nets, screens, and quarters for residual effect and (h) minimum lethal dosage of insecticides and optimum dosages for practical control.

In many of these problems little progress was made, in others valuable information was gained. It is hoped that those individuals who carried on various phases of the work will publish their findings.

F. FACTORS LIMITING EFFECTIVENESS OF PROGRAM

The following discussion is essentially a critical analysis of the faults apparent in the organization and operation of entomological work in the South Pacific Area. We realize fully that many of these faults are directly traceable to the urgency of the military situation and the necessity of prosecuting a campaign with inadequate personnel and equipment. Nevertheless, it is hoped that these comments will be received in the spirit in which they are presented, one of constructive criticism.

At all times there was need for competent research personnel, adequately equipped, who could devote their entire attention to investigational work. Medical General Laboratories were seldom available, and when present they were either uninterested or incapable of conducting the critical studies needed. Malaria Survey Detachments included many competent investigators but these individuals were invariably so overloaded with the urgent current problems connected with control operations that they had little time for carrying out long-time experiments. It would have been highly desirable, when sufficient personnel became available, to assign certain units exclusively to investigational work. This was actually done for a brief time on Guadalcanal. However, such an arrangement posed serious problems in personnel management. Most entomologists were anxious to participate in research work, and the younger men in particular, realizing that their professional future to some extent depended upon their demonstrated ability to carry out research projects, were eager to have equal opportunities in this respect. The problem would appear to be best solved by the organization of entomological units designed for investigational work and having no other duties.

The laboratory equipment of the Malaria Survey Detachment was primarily designed for parasitological work and the entomological equipment was noticeably inadequate. Such small but essential items as aspirators were never included. Literature was scanty, and current literature, or information as to its existence, was difficult to obtain. In order to carry on serious entomological work or even adequate routine survey work in isolated overseas posts, it was necessary to improvise much of the equipment.

There was constantly felt a need for entomological assistance from higher command echelons. Entomologists, or others with an understanding of the needs of field workers, could have been invaluable in channeling to the overseas units pertinent information, and in expediting the procurement of unusual items of equipment.

The shortage of personnel and equipment in the early phases of the campaign has been alluded to elsewhere. This situation was by no means peculiar to malaria survey activities, but was nevertheless at times critical in that phase of the work. At one time on Guadalcanal one officer and five enlisted men, with two "jeeps" for transportation, were responsible for the field survey work of approximately 45 square miles of territory, all of it new to the personnel and half of it

the newly occupied Tetere area, soon to become the staging area for a Marine Division. Within two weeks after the beginning of survey activities it was necessary to submit recommendations for projects to be undertaken by a Naval Construction Battalion, while at the same time preparing maps, establishing a laboratory, and guiding the efforts of the larviciding crew. This example, while extreme, was not considered unusual in the early phases of the Solomon campaign. The subsequent arrival of additional army personnel, adequately equipped with authorized transportation, fortunately corrected this situation.

A critical evaluation of officer personnel and their place in the malaria and insect control program is a difficult problem. While recognizing the need for over-all coordination of this program, we feel that in many instances the choice of Medical Corps Officers for this function was not necessarily a wise one except in theory. In actual practice this arrangement often meant that medical officers with little or no background in preventive medicine and medical entomology and hence unqualified to exercise broad judgment in the development of a program were supervising the activities of well trained and thoroughly competent entomologists and sanitary engineers. The prime prerequisites for successful planning and supervision of a malaria and insect control program are sound basic training and the ability to exercise good judgment. Experience showed that medical corps personnel did not always possess these attributes to the desired degree.

Normally the direction of the larvicidal program was considered a function of the sanitary engineer and in relatively few instances was this work directed by the entomologist. In general it is believed that the larvicidal program could have been handled more expeditiously by the entomologist since the field survey personnel under his direction were usually more familiar with breeding sites and larval populations than was any other group. Insect control, particularly the application of insecticides, is a normal function of entomologists for which many are especially trained. This arrangement would have been particularly desirable on those bases where the engineer was responsible for numerous draining, filling, and construction projects which involved the operation and maintenance of considerable heavy equipment; these activities usually required the major portion of the engineer's time and frequently led to inadequate supervision of the larvicidal program.

Little critical survey or control work could be accomplished under combat conditions. In retrospect, the desirability of undertaking survey activities in the first few days of a military operation seems questionable. Rather it is believed that control work at such times should consist largely of individual protective measures and such larviciding as can be done by the combined survey and control personnel. As the military situation permits regular survey activities can be initiated.

After combat ceased on many bases there was a tendency for incoming organizations to bivouac in previously unoccupied sections as well as for organizations previously present to move to apparently more desirable locations, thus expanding

the occupied area of the base and greatly increasing the territory to be controlled. On large bases such as Guadalcanal this promised to be a very serious problem until, at the request of the malaria and insect control organization, the island command eventually established a boundary limit beyond which organizations could not bivouac except in those cases where the location was dictated by tactical reasons. Even with this restriction there still existed factors which served to minimize control efforts. Combat teams often chose to hold maneuvers, usually involving night operations and patrols, outside the controlled area. Numerous individuals and small groups, for purposes of recreations, or rarely on business, journeyed into the uncontrolled portions of the island and stayed overnight or lingered after dark without adequately observing individual protective measures. On the basis of the first attacks, many cases of malaria could be traced to such injudicious trips to native villages or outposts.

MALARIA AND OTHER INSECT-BORNE DISEASES IN THE SOUTH PACIFIC CAMPAIGN 1942-1945

IV. PARASITOLOGICAL OBSERVATIONS ON MALARIA IN NATIVES AND TROOPS, AND ON FILARIASIS IN NATIVES

NORMAN D. LEVINE, PH.D.,¹ AND PAUL HARPER, M.D.²

The parasitology section of the Army-Navy Malaria and Insect Control Organization was headed by a commissioned officer parasitologist (Army Sanitary Corps or Navy H-V (S)), and included a senior non-commissioned officer in charge of the parasitology laboratory and 1 or 2 enlisted laboratory technicians. The parasitology section carried out malaria surveys among natives, white civilians, troops and Japanese prisoners to determine the incidence and geographical distribution of this disease and to check the efficacy of drug and other control measures. It carried out surveys to determine the incidence of filariasis and other parasitic diseases in natives and troops. It provided a microscopic diagnostic service for dispensaries, sick bays and other installations which were not equipped to do this work. The magnitude of the diagnostic service may be seen from the fact that in 1943 alone over 75,000 slides were read. In addition, the malaria diagnoses made by hospitals, sick bays and aid stations which read their own slides were checked periodically to ensure the reliability of their findings. The parasitologist participated in the malaria control training program and in other work of malaria control officers, such as inspection of troop areas for malaria discipline, and also collected and compiled statistical data on malaria incidence in military personnel.

This paper presents the results of surveys of malaria and filariasis in natives and observations on the species of *Plasmodium* found in troops.

A. SURVEYS OF NATIVES

The only information on malaria in the Solomon Islands before the war which was available to the Army and Navy Malaria and Insect Control Organization was the report of Sayers (1943), who had conducted a hospital at Munda, New Georgia and later at Vella LaVella from 1927 to 1934. During this period he treated 741 parasite-positive cases of clinical malaria, most of which came from a population of about 6000 in the New Georgia group of islands. He found *Plasmodium falciparum* in 44% of these cases, *P. vivax* in 32%, and *P. malariae* in 18%; the species was not determined in 6%. Mixed infections were present in 3% of the group. He found that during the first and second years of life, vivax malaria was the most common; that all three species were about equal in incidence from 3 to 5 years of age; that from 5 to 10 years of age, *P. falciparum* accounted for

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over half the cases, followed by *P. malariae* and *P. vivax*; and that in natives over 10 years of age, *P. falciparum* caused 82% of the cases, *P. vivax*, 12%, and *P. malariae*, 6%. Clinical malaria was uncommon in natives over 30 years old. In a spleen survey which he carried out among 365 children in the New Georgia group, he found a splenomegaly rate of 73%. Sayers found that the malaria season was from January to June, occurring during and immediately following the rainy season. The most malarious months were April, May and June, the incidence of the disease being highest in June.

In order to determine the incidence of malaria and filariasis among the natives on the islands where troops were stationed, and hence the extent of the danger to troops which they represented, numerous surveys were carried out by personnel of the malaria survey units in the theater. The data presented herein were compiled from reports of the parasitologists of these units which were available at the Guadalcanal Malaria Control Headquarters and at the Theater Headquarters. Only a small proportion of these surveys was carried out by the authors. Since the authors are not now in possession of the original reports, it is impossible to give a complete list of the names of those who carried them out.³ The present report gives an overall picture of the findings in the South Pacific; it is expected that the results of special studies will be reported separately by other members of the Malaria and Insect Control Organization.

These surveys were made during a two-year period from August 1942 to August 1944. All persons examined in the Solomon Islands and Bismarck Archipelago were Melanesians. In the New Hebrides, most of those examined were Melanesians, but also included is a number of Indo-Chinese who had been imported as laborers.

The blood examinations on which the data are based were made on Giemsa-stained, thick smears. In some surveys, thin smears were also made for use if species identification was uncertain in the thick smear. Only a single smear was made from each individual.

1. Malaria in Natives

Incidence of malaria. In Table I are given the incidences of malaria on the islands studied. There is no malaria on New Caledonia, Samoa and Figi, since no *Anopheles* occurs on these islands. The figures in this table indicate persons in whose blood parasites were found, not clinical malaria cases, and are thus not comparable with Sayers' figures. These surveys were made on persons of all ages, but mostly on adults. They were made at all seasons of the year, but the data are inadequate to demonstrate seasonal variations.

³ As an example of the number of persons involved, the surveys reported from Guadalcanal, Savo, Florida, Malaita, San Cristobal and Green Islands were performed by the following officers, assisted by numerous enlisted men whose names are not available: John G. Arnold, Jr., Curtis A. Beerman, Virginus E. Brown, Joseph H. Denton, Jr., Henry W. Deurloo, Paul Harper, Norman D. Levine, Otto L. Munch, R. C. Page, David Schiffer, Ralph J. Schlosser, Louis E. Schopick, Carlos D. Speck, Robert T. Stevenson, and Monroe M. Vincent.

The surveys on natives of Malaita and San Cristobal were carried out on adult males who had been recruited as laborers by the British Protectorate Government, and who were living in labor camps on Guadalcanal at the time of examination. The surveys of Guadalcanal natives are divided into those made on a similar group of adult male laborers and those made on persons of both sexes and all ages living in villages outside the troop area. The malaria parasitemia rates of these latter two groups (52% for the villagers and 10% for the laborers) were markedly different. Part of this difference was due to the fact that the incidence of malaria decreases with increasing age, and part probably to the fact

TABLE I
Incidence of malaria parasitemia among South Pacific natives

ISLAND	NUMBER OF SURVEYS	NUMBER EXAM.	PARASITIC INDEX (%)	
			Average	Range
New Hebrides				
Efate.....	12	4000	10	5-50
Espiritu Santo.....	6	4000	13	7-57
Solomons				
Guadalcanal				
Villagers.....	15	451	52	6-74
Laborers.....	1	106	10	
Total.....	16	557	44	6-74
Savo.....	4	427	26	11-39
Florida.....	1	37	65	
Malaita (Laborers).....	6	286	11	4-19
Russells	3	193	64	57-73
Bougainville.....	1	500	10	
San Cristobal (Laborers).....	1	57	7	
Treasury.....	1	37	16	
Bismarck Archipelago				
Green*.....	2	350	45	43-48
Emirau.....	2	313	16	12-21

* Smears made one month after evacuation to Guadalcanal.

that in the selection of laborers by the recruiting officers, only vigorous, healthy-appearing individuals were accepted. So far as is known, none of these men had received atabrine prior to examination. The parasitemia rates of the Malaita and San Cristobal laborers (11% and 7%), respectively were similar to those of the Guadalcanal laborers, which suggests that these rates cannot be taken as indicative of the true malaria rates among villagers on these islands.

The survey on natives from Green Island was carried out on Guadalcanal. This group had been evacuated to Aola Bay on Guadalcanal, about 25 miles east of the troop area, and was examined about a month after its arrival there. It had very little contact with Guadalcanal natives prior to examination.

Plasmodium species distribution. In Table II are given the species of *Plasmodium* encountered in the surveys. The most common species on all islands

except Emirau was *P. vivax*. On the other islands, this species was identified in from 44% to 88% of the positive smears (exclusive of mixed infections). The next most common species was *P. falciparum*. On Emirau it was the commonest, being found in 45% of the positive slides, while on the other islands its incidence ranged from 6% to 37%. *P. malariae* was encountered on Espiritu Santo, Guadalcanal, Savo (more prevalent than *P. falciparum* on this island), Florida, Russells, Green and Emirau, but not on Efate, Malaita, San Cristobal or Treasury. Its incidence on the different islands ranged from 0.5% on Espiritu Santo to 25% on Emirau.

Relation of age to incidence of malaria. In Table III are given the data available on the relation of age to the incidence of *Plasmodium parasitemia* in natives

TABLE II
Plasmodium species distribution among South Pacific natives

ISLAND	NUMBER OF SURVEYS	NO. OF POS. SMEARS	PLASMODIUM SPECIES (%)				
			vivax	falcip.	malar.	Mixed	Under- term.
New Hebrides							
Efate.....	5	205	66	25		4	4
Espiritu Santo.....	5	486	57	37	0.5		5
Solomons							
Guadalcanal.....	14	200	60	18	12	6	4
Savo.....	3	103	56	13	20	11	
Florida.....	1	24	79	12	8		
Malaita.....	5	29	67	21			12
Russells.....	3	123	41	30	4		25
San Cristobal.....	1	16	88	6			6
Treasury.....	1	6	84	16			
Bismarck Archipelago							
Green*.....	1	132	46	14	1	8	31
Emirau.....	1	25	29	45	25		

* Smears made one month after evacuation to Guadalcanal.

of Guadalcanal, Savo, Green, and the Russell Islands. Three age groups (birth to 5 years, 6 to 15 years, and over 15 years) were selected for purposes of comparison in the Guadalcanal and Savo surveys. In the Green Island survey the younger two age groups were birth to 3 years and 4 to 15 years, while in the Russell surveys the age groups are 0-4 years, 5-14 years, and over 14 years. The ages in these surveys were estimated by the examiners. It is seen that the parasitemia rate decreased markedly with increasing age. Among Guadalcanal natives, for example, the rate in the 0-5 age group was 91%; in the 6-15 age group 72%; and in those over 15 years, 38%. The rates for the other islands are similar.

The incidence of the different species varied with age, also. On Guadalcanal, *P. vivax* was the most common species in all age groups; *P. falciparum* was the most common species in the 0-5 group, but its incidence increased exponentially with age, paralleling decreases in the incidence of *P. malariae*, so that the latter

MALARIA IN THE SOUTH PACIFIC CAMPAIGN

species had the lowest incidence in the two older age groups. Trends are not so clear in the other surveys.

TABLE III
Relation of age to incidence of malaria among South Pacific natives

AGE GROUP	NUMBER OF SURVEYS	NO. OF PERSONS	% POS. SMEARS	PLASMODIUM SPECIES (%)				
				vivax	falcip.	malar.	Mixed	Unde-term.
0-5 yrs.	5	44	91	47	13	25	15	0
Guadalcanal.....	1	27	96	50	0	31	20	0
Savo.....	1	33	88	69	7	0	10	14
Green*.....	1	33	88	69	7	0	10	14
Russells†.....	3	59	92	54	30	4	7	6
6-15 yrs.	5	51	72	54	32	11	5	0
Guadalcanal.....	1	28	75	67	5	19	10	0
Savo.....	1	96	56	68	9	0	13	9
Green*.....	1	96	56	68	9	0	13	9
Russells†.....	3	45	76	42	31	3	3	22
Over 15 yrs.	5	96	38	67	28	3	0	3
Guadalcanal.....	1	88	17	67	7	27	0	0
Savo.....	1	175	28	8	24	2	0	65
Green*.....	1	175	28	8	24	2	0	65
Russells†.....	3	89	36	25	37	6	0	31

* Smears made one month after evacuation to Guadalcanal. Age groups in this survey are 0-3 yrs., 4-15 yrs., and over 15 yrs.

† Age groups are 0-4 yrs., 5-14 yrs., and over 14 yrs.

TABLE IV
Splenomegaly among South Pacific natives

ISLAND	NUMBER EXAM.	% WITH SPLENOMEGALY
New Hebrides	110	61
Efate.....	101	57
Espiritu Santo.....		
Solomons	258	73
Guadalcanal.....	37	70
Florida.....	219	57
Malaita (Laborers).....	193	66
Russells.....	500	75
Bougainville.....	219	57
San Cristobal (Laborers).....	37	34
Treasury.....		
Bismarck Archipelago	542	65
Green.....	263	85
Emirau.....		

Splenomegaly rates. The incidence of splenomegaly among natives of all ages on the different islands is given in Table IV. Spleen rates varied from 34% on Treasury Island to 85% on Emirau. In some surveys, spleen classification was recorded. These figures are given in Table V.

2. *Filariasis in Natives*

It has long been known that there is a high incidence of filariasis due to *Wuchereria bancrofti* in Samoa and Fiji, but very little was known about its incidence in the Solomon Islands. Filariasis surveys were carried out by parasitologists of the malaria survey units, and also by a special Navy Filaria Survey Unit

TABLE V
Spleen classifications among South Pacific natives

ISLAND	NUMBER OF SURVEYS	NO. OF PERSONS	PER CENT SPLENOMEGALY	SPLEEN CLASSIFICATION (%)				
				PDI	+	++	+++	++++
Efate..	1	110	61	6	22	39	28	4
Guadalcanal.....	6	244	85	3	27	40	27	3
Florida.....	1	37	70		46	35	12	8
San Cristobal.....	1	219	57		23	31	34	12
Russells.....	3	193	66	9	26	41	16	6

TABLE VI
Incidence of microfilariae among South Pacific natives

ISLAND	INDIGENOUS NATIVES*, WUCHERERIA BANCROFTI		INDO-CHINESE, WUCHERERIA MALAYI	
	No. exam.	% positive	No. exam.	% positive
Samoan Area.....	?	24†		
Fiji.....	300	30†		
New Caledonia.....	247	4†		
Loyalty.....	270	12†		
New Hebrides				
Efate.....	110	17†		
Espiritu Santo.....	?	22†	?	9
Solomons				
Guadalcanal.....	2500	22†		
Bismarck Archipelago				
Emirau.....	62	39†		

* Melanesians on all islands except Samoa; Polynesians in the Samoan area.

† Non-periodic.

‡ Nocturnal periodicity.

whose officers were E. E. Byrd and L. S. St. Amant. An abstract of the work of this group has been published by Byrd (1945). The results of these plus the other filariasis surveys are given in Table VI. The figures show the percentages of individuals examined in whose circulating blood microfilariae were found in Giemsa-stained, thick blood films. Children were not included in these surveys.

No periodicity was found in the appearance of *W. bancrofti* microfilariae in the blood in Samoa, Fiji, New Caledonia or the Loyalty Islands, but nocturnal periodicity was observed in the Solomons, New Hebrides, and Emirau. This could be correlated with the habits of the vectors. In Samoa and Fiji, the

principal vector of filariasis is the day-feeding mosquito, *Aedes scutellaris pseudoscutellaris*; in the Solomons and New Hebrides it is probably the night-feeding mosquito, *Anopheles farauti laveran*.

With one exception, *Wuchereria bancrofti* was the only filariid species found in these surveys. On Espiritu Santo, however, the Navy Filaria Survey Unit found *W. malayi* microfilariae in 9% of the Indo-Chinese who had been imported as laborers under the Condominium Government. This species was not present in the Melanesians, and no vector for it was found on the island. It is apparent that these infestations had been acquired in Indo-China prior to migration to the New Hebrides.

B. PLASMODIUM SPECIES IN TROOPS

The malaria encountered among troops in the South Pacific Area was caused almost entirely either by *P. falciparum* or *P. vivax*. Cases due to *P. malariae* were rare.

The predominant species showed a characteristic change in malarious islands of this area where adequate studies were made. Early in the campaign, falciparum infections predominated, but as control measures improved in efficiency and as the general malaria rate decreased, the proportion of vivax infections steadily increased. This is shown in figure 1, compiled from data on laboratory-confirmed cases on Guadalcanal.

In January, 1943 there were twice as many cases of falciparum as of vivax malaria. The incidence of the two species was almost equal in July; and in January, 1944, there were twenty times as many vivax as falciparum cases. It should be noted here that the total malaria rate for Guadalcanal for January, 1943 was 1042 per thousand per annum; for July, 1943 it was 608 per thousand per annum; and for January, 1944 it was 200 per thousand per annum.

Several factors were associated with this sequence of events. In January, 1943 mosquito control and personnel protection were still inadequate, and a condition of hyperendemicity existed. Under these conditions, a large percentage of the falciparum cases at this time were probably mixed infections in which *P. vivax* was suppressed. That this antagonistic action is common has been shown by Boyd and others. During this early period, the malaria cases were almost all primary, but as time went on an increasing percentage were relapses. Vivax malaria has a very much greater tendency to relapse than does falciparum malaria, and this factor acted to increase the proportion of vivax cases progressively. This shift in species distribution presented a seed-bed containing an increasingly higher proportion of vivax cases to the mosquito vectors; consequently they transmitted a progressively higher percentage of new vivax cases as time went on. In addition, as malaria control became effective and decreased the transmission rate, double infections became less common, so that vivax malaria was no longer masked as much as before by falciparum malaria. Improvement in atabrine discipline also contributed to the decline in falciparum malaria, since this drug has a curative effect in the full sense of the word against *P. falciparum* while it only results in a clinical cure of *P. vivax* malaria. It suppresses both species.

Parasite species incidence after removal of infected troops to non-malarious areas. When infected troops were transferred from hyperendemic territory to non-malarious areas for rehabilitation, *P. vivax* rapidly became the dominant species.

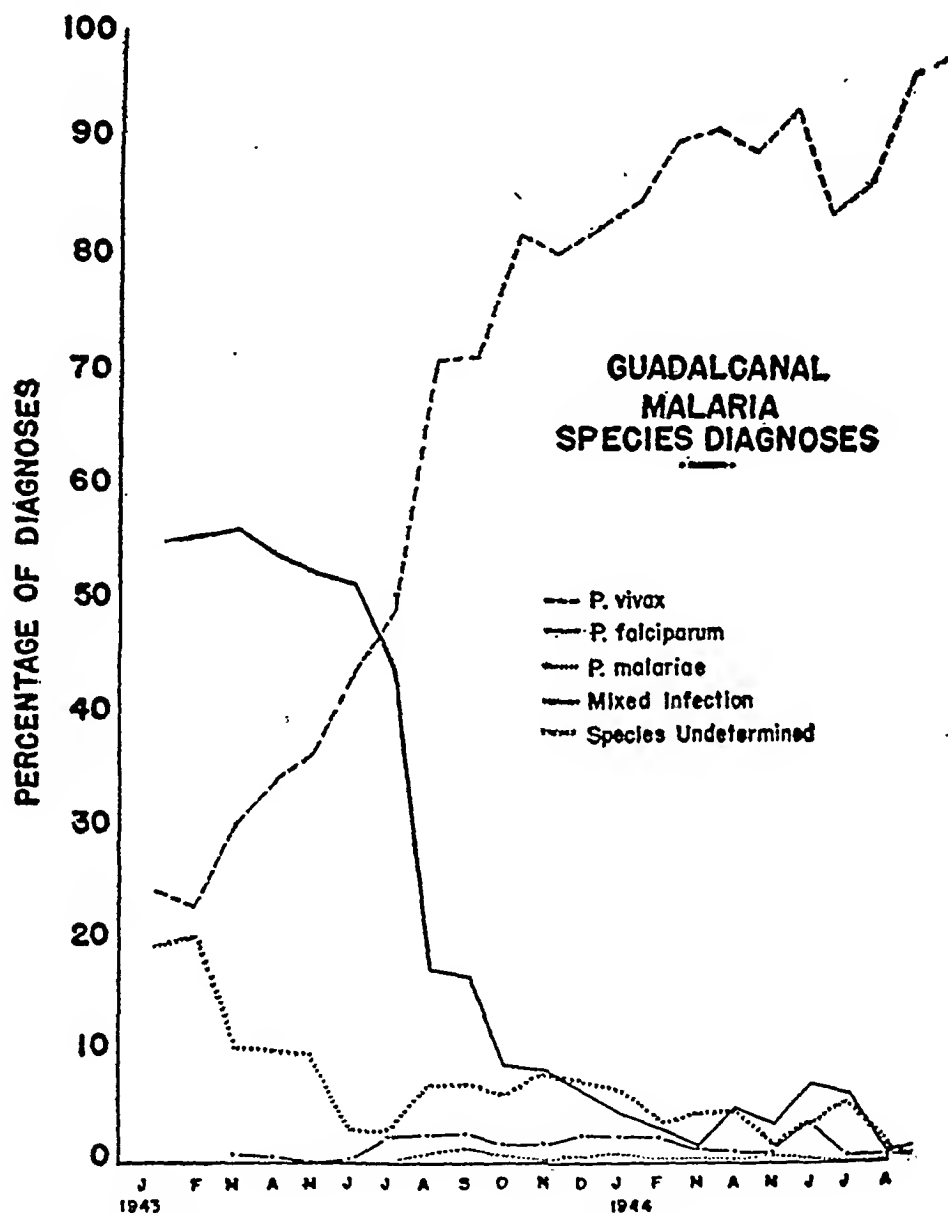


FIG. 1. SPECIES OF PLASMODIUM AMONG TROOPS ON GUADALCANAL

It was the general experience of hospitals in rear bases in the South Pacific and in the United States that many individuals who had falciparum malaria on Guadalcanal or other malarious bases had vivax malaria when they relapsed later on.

Table VII shows the progressive increase in percent of vivax malaria in more than 8000 "first attacks of malaria in New Zealand" occurring in the 2nd Marine

Division, which was moved from Guadalcanal to New Zealand during early February, 1943. These "first attacks" were the initial ones recorded in New Zealand, and were not necessarily primary attacks since many of these patients had had malaria on Guadalcanal.

The percentage of *P. vivax* infections increased from 67 in February to 99 in June, while that of *P. falciparum* decreased from 27 in February to 0 in June. The figures given for January are those of Guadalcanal as a whole, and have been inserted for purposes of comparison. It should be mentioned that all members of this division had mass therapy with either quinine or atabrine enroute to New Zealand.

The disappearance of *P. falciparum* malaria a few months after leaving a malarious area occurred in many well-studied groups.

TABLE VII
Plasmodium species in troops following departure from malarious area

PLASMODIUM SPECIES	PERCENTAGE OF TOTAL CASES					
	Guadalcanal*, Jan. 1943	New Zealand†				
		Feb.	Mar.	Apr.	May	June
<i>vivax</i>	24	67	74	92	96	99
<i>falciparum</i>	55	27	12	4	1	0
Mixed.....	0	4	9	3	1	0
Undetermined.....	19	2	4	1	2	1

* All troops.

† 2nd Marine Division.

C. DISCUSSION

It should be mentioned that percentage incidences of malaria parasitemia and of microfilariae listed in the various native surveys are probably not strictly comparable. In the malaria surveys, differences in age composition of the surveyed groups strongly affect the resultant rate, since it is much higher in children than in adults. In surveys made by different individuals, a certain amount of variation is to be expected because of personal factors, such as the amount of blood used in making the smear, the amount of time spent in searching each smear, and the efficiency of the microscopists. Nevertheless, the figures given here are considered sufficiently reliable for practical purposes.

Malaria is obviously hyperendemic among the natives of the Solomons and New Hebrides. The very high parasitemia rates in children, the lower rates in adults, and the high spleen rates all indicate this fact. Filariasis, too, is a disease to be reckoned with, not only in Samoa and Fiji, but also in the Solomons and New Hebrides.

D. SUMMARY

1. Malaria parasitemia rates among natives of the Solomon Islands, New Hebrides and Bismarck Archipelago as determined by malaria survey units in

the South Pacific between 1942 and 1944 are presented. They show that malaria is hyperendemic in this area.

2. *Plasmodium vivax* was the most common species encountered in these surveys. *P. falciparum* was second in incidence, and *P. malariae* third. Relative incidences of the three species varied on the different islands.

3. The incidence of splenomegaly among natives varied from 34% to 85% on the different islands, but on most was between 50% and 75%.

4. The incidence of microfilariae of *Wuchereria bancrofti* among the natives varied from 4% on New Caledonia to 39% on Emirau. The rate was 22% on Guadalcanal and Espiritu Santo, 24% in the Samoan area, and 30% in Fiji.

5. Nocturnal periodicity of microfilariae was noted on Guadalcanal, Espiritu Santo and Emirau. There was no periodicity in Samoa or Fiji.

6. *Wuchereria malayi* was found in 9% of imported Indo-Chinese laborers on Espiritu Santo, but not among the natives on any of the islands surveyed.

7. In the early part of the South Pacific campaign, the predominant type of malaria among troops was caused by *P. falciparum*. The falciparum-vivax ratio decreased progressively, so that in the latter part of the occupation vivax malaria was far more common than falciparum. This shift was associated with a marked decrease in the general incidence of malaria.

8. Upon removal of troops from a malarious area, *P. vivax* rapidly became the predominant cause of malaria.

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THE EPIDEMIOLOGY OF SCHISTOSOMIASIS JAPONICA IN THE PHILIPPINE ISLANDS AND JAPAN. III. SURVEYS OF ENDEMIC AREAS OF SCHISTOSOMIASIS JAPONICA IN JAPAN

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Surveys of the endemic areas of schistosomiasis in the Sixth and Eighth Army Commands on Honshu and Kyushu were carried out between October 29 and December 3, 1945, with the view of obtaining information concerning the extent of these areas and the securing of other data which might be pertinent to the prevention of the disease in military personnel.

Prior to its departure from the United States, the commission had not foreseen that it would be called upon for studies of schistosomiasis in the Japanese islands, and consequently had compiled no information on the status of the disease in Japan. Furthermore, during its tour of duty in the Philippine Islands, it had no access to literature on the subject. Consequently, when the commission arrived in Japan on October 25, 1945, it had only a general knowledge concerning the endemic areas of this disease in that country. Because of the relatively short time allotted for the surveys in question, opportunity was not available for a search of the Japanese literature. Since the return of the commission to the United States, effort has been made to review this literature. It has been found that many of the original articles are not available in this country; moreover, difficulty has been encountered in securing translations of the Japanese text of those papers which are accessible. For these reasons, this report is written with only casual reference to the existing literature and is therefore without the usual historical review. However, it is believed that the information obtained from health officers and others is of more recent origin and is more complete than that available in any published account. Titles of many of the older Japanese papers are given by Faust and Meleney (9).

It is our belief that the information in this report is substantially correct. However, language difficulties often constituted a formidable barrier in securing essential data, and some of the field work was done with the aid of interpreters who had no knowledge of the disease or its terminology. For these reasons, in the case of information furnished by others, it is possible that errors of interpretation have unavoidably crept into the written account.

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CONTACT WITH JAPANESE GOVERNMENT OFFICIALS

Immediately on arrival of the commission in Tokyo, contacts were made with Japanese health officials through the courtesy of Colonel C. F. Sams, Chief, Public Health and Welfare Section, SCAP. Available information concerning the present status of the endemic areas of schistosomiasis in Japan was obtained at several conferences with Prof. K. Nobechi, Chief, Division of Preventive Medicine, Institute of Public Health, Dr. N. Tatebayashi, Chief, Section of Communicable Diseases, Ministry of Welfare and Social Affairs, Dr. Nobutaro Ishii, Chief, Research Laboratory of Parasitology, Government Institute for Infectious Diseases, and Prof. K. Nagano, Chief, Parasitology Department, Kitasato Institute for Infectious Diseases. General information concerning the location of the endemic foci of the disease was obtained from these officials, but they were able to furnish little information of a specific nature concerning morbidity and mortality rates, the incidence of infection, or the distribution of the disease in the several known endemic areas. Tables showing percentage of egg carriers in various Prefectures for the years 1925 to 1928 were supplied, but the number of examinations was relatively small and the data were not broken down into counties or townships.

Some of the Prefectural and local health authorities were able to furnish valuable data, and where information of this sort was secured, it has been incorporated in this paper.

GENERAL DISTRIBUTION OF *Schistosoma japonicum* IN JAPAN

There are five recognized endemic areas of schistosomiasis in Japan (Fig. 1), viz: The Tone River area in Chiba and Ibaraki Prefectures, northeast of Tokyo; the Kofu area, centered around the city of that name in Yamanashi Prefecture, approximately 70 miles due west of Tokyo; the Numazu area in Shizuoka Prefecture approximately 75 miles southwest of Tokyo on Suruga Bay; the Fukuyama area, immediately to the north of the city of Fukuyama, and covering parts of Hiroshima and Okayama Prefectures; and the Kurume area, surrounding the city of Kurume, island of Kyushu, and covering parts of Saga and Fukuoka Prefectures.

In addition to these recognized endemic areas, schistosomiasis has been reported in other parts of Japan. For instance, War Department Technical Bulletin, TB MED 160 (1) lists egg carriers in Tochigi, Aomori, and Fukui Prefectures, in addition to those in Prefectures in which the disease is known to be endemic. The Commission endeavored to secure definite information concerning the occurrence of autochthonous cases of the disease in the three above-mentioned prefectures. Japanese health authorities in Tokyo were of the opinion that the finding of egg carriers in Tochigi and Aomori Prefectures is probably indicative of imported cases, and that the disease is not endemic in these prefectures. The same opinion was given in connection with the Fukui Prefecture. However, attention was called to the data in TB MED 160 which lists 168 *Schistosoma* egg carriers in villages in Fukui Prefecture among 2,000 individuals examined in 1937-38. A search of the records in the Ministry of Welfare and Social Affairs

disclosed reports for 1940 of 4 carriers in 110 examinations in Fukui City and 3 carriers in 70 examinations in villages. In view of the conflicting evidence regarding this area, at the request of the commission, the Ministry telegraphed the Health Officer of Fukui Prefecture for specific information concerning the status of the disease in that prefecture. That official replied that there were no cases of schistosomiasis in that prefecture at that time.

Schistosomiasis japonica was at one time endemic along the Edo and Arakawa

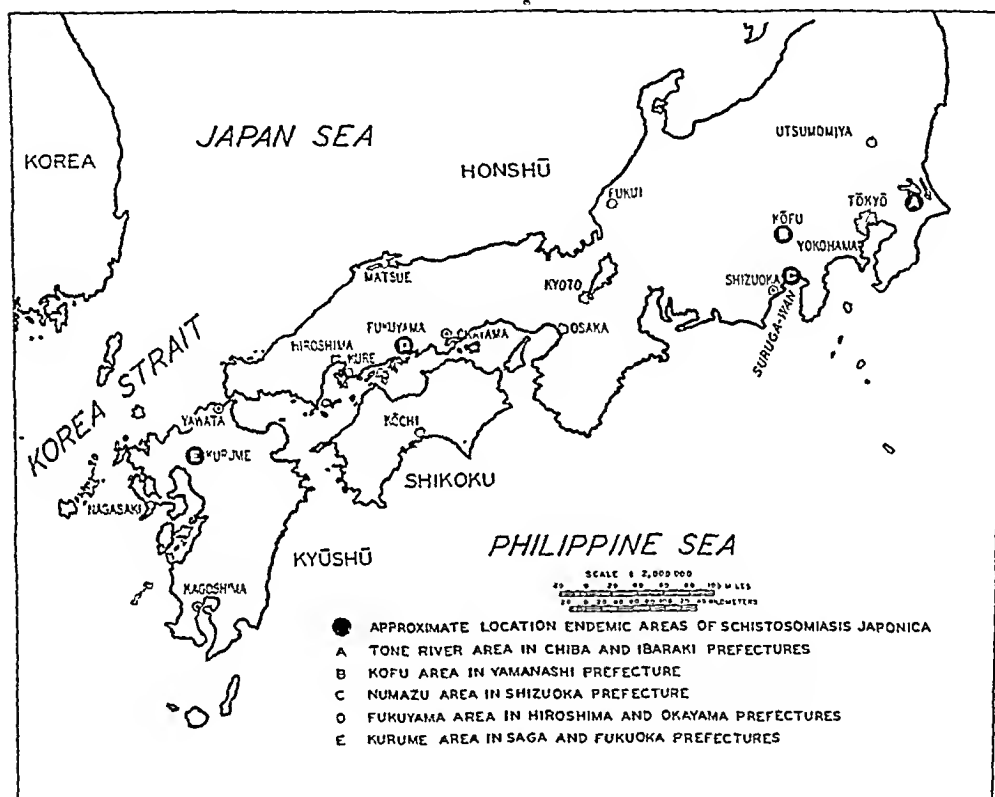


FIG. 1. SOUTHERN HONSHU AND KYUSHU SHOWING APPROXIMATE LOCATION OF ENDEMIC AREAS OF SCHISTOSOMIASIS IN JAPAN

Rivers in Tokyo Prefecture (2). However, Japanese health officials stated emphatically that these endemic foci had been eradicated.

The figures reproduced with this paper carry outlines of the present boundaries of the various endemic areas. Such boundaries are predicated on the information furnished the Commission by Japanese health authorities and others and on results of the Commission's own investigations. For military purposes, effort was made to place these boundaries at safe limits. It is possible that the disease does not actually exist in all of the territory included within the boundary lines. However, any lapses in this respect could only be rectified through more prolonged and more detailed surveys.

METHOD OF CONDUCTING SURVEYS

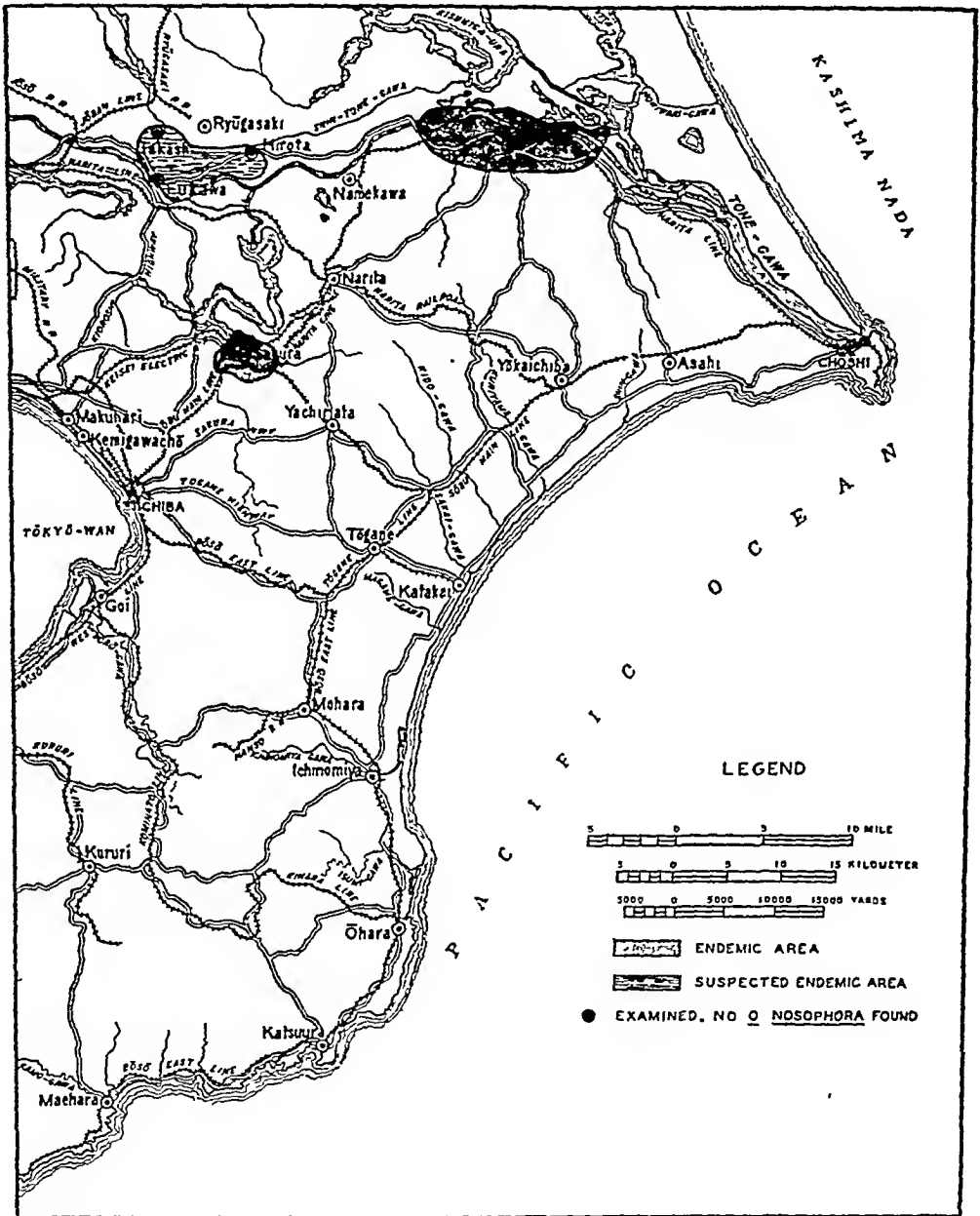
On entering an endemic area, as much information as possible concerning the status of the disease was obtained from prefectural and local health authorities and from practicing physicians. Based on this information, certain schools in various parts of the area were selected, and a representative number of stool samples obtained from children in the higher age groups (8 to 15 years) for microscopic examination for schistosome eggs. Surveys were then conducted in various parts of the area for *Oncomelania (Katayama) nosophora*, the snail intermediate host of *Schistosoma japonicum*. When the snails were found, sufficient numbers were collected for transport to the laboratory for examination for incidence of infection.

All stool examinations conducted by the commission were carried out with the use of the plain sedimentation technique. This technique had previously been found to be the most efficient one for practical use in the field, through research carried out by members of the commission on Leyte and other islands. The technique consisted in taking five grams of feces, stirring the sample in water, straining the material through four layers of surgical gauze into a 250 cc. conical urinalysis flask, allowing the material to sediment, and pouring off the supernatant fluid. At least three decantations were made. One drop of the final sediment was placed on a slide and examined under the microscope. In the examination of school children in Japan, the commission followed the practice of examining two 22 x 22 mm. cover glass preparations or one 22 x 40 mm. cover glass preparation from each sample. Data were recorded for all helminth parasites, but no examination was made for protozoan parasites. It should be noted that the findings of helminth eggs other than those of *Schistosoma japonicum* were made incidently; when the latter eggs were found on a slide, the slide was discarded and no further search made. It is probable under these circumstances that some cases involving infection with other helminths were missed, and that the incidence figures for these helminths are actually lower than the true incidence. Since a single sedimentation examination will undoubtedly fail to disclose some light infections, the incidence figures are no doubt actually higher than those recorded.

SURVEYS IN THE TONE RIVER AREA, CHIBA AND IBARAKI PREFECTURES

General description and extent of infection. The infection in the Tone River area occurs in several small foci, apparently quite far removed from one another, without, insofar as we were able to learn, any connecting links (Fig. 2). One area is centered around the city of Sawara and includes parts of Toyoura, Tsunomiya, Toyoshima, and Higashiōto Townships (Mura), Katori County (Gun), and the Kita-Sawara section in Ibaraki Prefecture across the Tone River from Sawara. This area (Fig. 3) measures approximately 13 miles from east to west and is about 5 miles in extent from north to south. Another area surrounds the city of Sakura and measures approximately 2 x 4 miles. A third area, concerning which there is some doubt at present, lies in Ibaraki Prefecture, extending east and west along the Tone River from Fukawa and northward as far as Mae-

shinden. Dr. Y. Utagawa, Chief of the Public Health Section of Ibaraki Prefecture, furnished information that the disease was possibly endemic in the Tone



River Valley as far west as Sakai, which is approximately 13 miles northwest of Torite (Fig. 2). Because of the lack of time, opportunity was not had to survey these last two areas. More recently, Capt. Louis J. Olivier, Sn. C., 443d Malaria

Survey Detachment, has advised that he has located a focus of infection at Kōya, which is about 1.5 miles south of Moriya (Fig. 2) in Kita-Sōma County, Ibaraki Prefecture.

At the time of our surveys, small scale (1:50,000) maps of these areas were not obtainable, and considerable difficulty was met in securing information from health authorities concerning definite boundaries of the endemic areas. How-

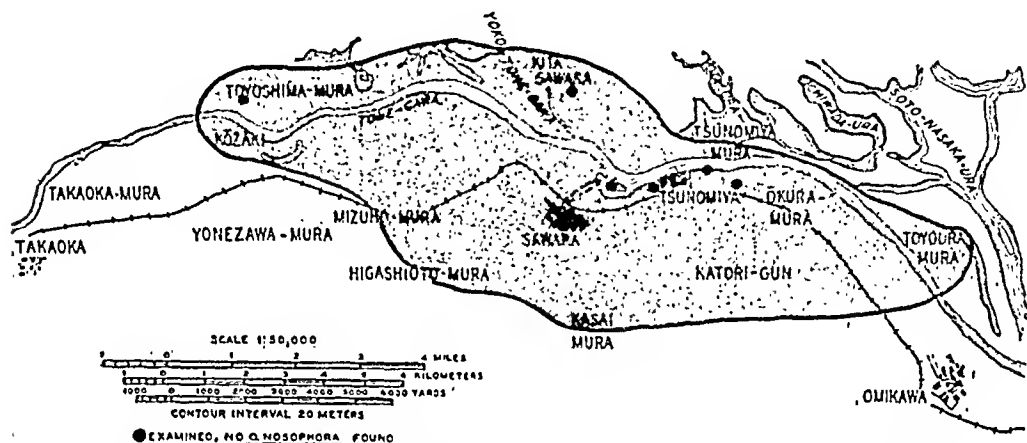


FIG. 3. ENDEMIC AREA IN THE VICINITY OF SAWARA, CHIBA AND IBARAKI PREFECTURES

TABLE 1

Results of schistosomiasis surveys carried out in the Tone River area by Health Department, Chiba Prefecture; entire population of areas surveyed

COUNTY	TOWN OR VILLAGE	YEAR	NO. PERSONS INFECTED	PER CENT PERSONS INFECTED
Imba	Nego, Chiyoda, Wada, Sakura, Asahi, Uchisato			
Higashikatsushika	Fuse, Tanaka, Abiko	1925	167	0.9
Imba	Shisui, Uchisato, Rokugō	1928	31	0.4
Imba	Usui, Aso, Shinu, Kōzu, Haku, Ajiki	1929	1	0.01
Katori	Niijima, Sawara, Tsunomiya	1934	31	0.4
Katori	Higashiōto, Sawara, Okura, Tsunomiya, Toyoura	1935	21	0.3

ever, it is quite possible that these zones are more extensive than present knowledge indicates, and that other endemic foci occur elsewhere in the Tone River Valley. At present the whole valley of that river in Chiba and Ibaraki Prefectures must be regarded as a suspected endemic area, although the infection rate in most of the known centers is very low.

Certain data were obtained from Dr. S. Murata, Chief of the Public Health Section, Chiba Prefecture Office, concerning schistosomiasis surveys in the Tone River area. Table 1 gives information concerning surveys made of the entire

examined in the four schools. This represents an infection rate of 0.8 per cent. This information together with that obtained from health authorities indicates that schistosomiasis japonica is still present in the Sakura community and in the Sawara area even though the incidence is at a very low level compared to that in other endemic areas in Japan.

Ascaris lumbricoides showed the highest incidence of any intestinal helminth, since 223, or 57.2 per cent, of 390 individuals were found infected. The incidence of hookworm infection was considerably lower, followed by that of *Trichuris trichiura*. *Hymenolepis nana* was found in 2 cases, *Clonorchis sinensis* in 13 cases, and *Metagonimus* sp. in 2 cases. In a number of instances, eggs of *Trichostrongylus orientalis* were encountered; however, the eggs of this parasite were first mistaken for those of free-living nematodes, and the actual number of infections was therefore not recorded.

Snail collections in the endemic areas in Chiba and Ibaraki Prefectures. Search was made for the snail intermediate host of *S. japonicum* in rice fields, drainage ditches, and in the Imba-Numa swamp northwest of Sakura for a distance of approximately one mile beyond the town. Several species of snails were found, but no specimens of *Oncomelania nosophora* were recovered. Snail surveys were conducted also in Toyoshima Township and in the Kita-Sarawa area, as well as on an island in the Tone River north of the city of Sawara. Snails of the genera *Parafossarulus* and *Melania* were found, but no specimens of *O. nosophora* could be located. Failure to find the snail intermediate host in areas adjacent to towns and villages in which the disease was demonstrated to occur indicates that it is only sparsely distributed throughout the endemic zones. This is in contrast to most of the other areas surveyed by the commission in Japan in which little difficulty was encountered in collecting the snail in regions in which the disease occurs. The low incidence rate of schistosomiasis in the Tone River area is no doubt associated with the sparse distribution of the snail intermediate host. This scarcity cannot be associated with control measures since little work of this nature has been carried out in this region. A limited effort was made in this direction between 1928 and 1931 when lime was applied to the irrigation ditches and a flame thrower used on the sides of the ditches and canals in Higashikatsushika County around the villages of Tanaka, Tomise, and Abiko, and near the village of Nego, Imba County. However, the work was not continued and probably had little influence on the distribution of the snail.

Morbidity and mortality in the Tone River area. Little specific information was available on these points. Local physicians informed us that a flood inundated a large part of the Tone River Valley in July, 1927, and a large number of *O. nosophora* were washed into the Sawara area. Within two months, 120 cases of schistosomiasis developed, all of which were acute and characterized by typical schistosome dermatitis and fever. Three years later (1930), a similar flood occurred and six cases developed at Kessa, a small town in Ibaraki Prefecture, about 5 miles up the Tone River from Sawara. Again in 1939, the area was flooded with the development of 21 acute cases in and near Sawara. In 1943, two acute cases developed in Moriyama, a small village about 12 miles down the

Tone River from Sawara. As noted in table 2, no positive findings were obtained from school children in this village.

SURVEYS IN THE KOFU AREA, YAMANASHI PREFECTURE

The only information which the Commission could obtain from national health authorities concerning the incidence of infection in the Kofu area prior to its investigation was as follows:

NUMBER OF PERSONS EXAMINED					PER CENT POSITIVE
1925	1926	1927	1928	Total	
698	491	630	203	2,025	6.8

At Kofu, conferences were held with Dr. M. Ienaga, Chief, Public Health Section, Yamanashi Prefecture, and Dr. Saburō Sugiura, who operated a private hospital at Showa-Mura, within the endemic area. Mr. Y. Miyoshi, Head, Schistosomiasis Control Office, Yamanashi Prefecture, furnished much valuable information and accompanied the party during its investigations.

General description and extent of infection. The endemic area of schistosomiasis in the Kofu basin comprises approximately 93 square miles (Fig. 4). The average altitude of the area is about 400 meters, but cases of the disease and the snail intermediate host have been found at Mutsusawa, 600 meters above sea level. This area has long been known as an endemic center of the disease, in fact even before the nature of the disease was determined and its etiology established. It was here in 1904 that Katsurada discovered the parasite, *Schistosoma japonicum*.

In cooperation with the national government, a control program on schistosomiasis was inaugurated in 1943 with an appropriation by the Prefectural government of 1,000,000 yen over a three-year period. In addition, the national government pledged 130,000 yen per year for the work.

At the time of the survey, the endemic area comprised three sections, viz: (1) A so-called control area in which banks of streams and rice fields were treated in 1942 with calcium cyanamide at the rate of about 500 pounds per acre; (2) an area under control in which application of the chemical was made in 1944; and (3) an area in which no chemical had been applied. Calcium cyanamide, known to the Japanese as "lime nitrogen," was one of the chemicals tested by the commission against *Oncomelania quadrasi*, the intermediate host of *Schistosoma japonicum* in the Philippine Islands (3). It was found to be very effective against this species of snail when applied at the rate now being employed in the Kofu area. In this area, application is made about 2 months before the rice is planted in May. Since calcium cyanamide has a high fertilizing value, there are two distinct advantages to its use.

The commission was told that a material reduction had been made in the incidence of schistosome infection in the control area, and that some such reduction had been effected in the area under control, although it had not been possible to make more than one application of the chemical because of a shortage of

supply. The original plan for control called for the application of the chemical to the endemic area each year, and undoubtedly it would be much more effective if repeated at intervals.

As part of the control program, stool examinations are carried out in four control stations which employ a total of 15 female technicians. These technicians are given training for 1 month and then work under the close supervision of

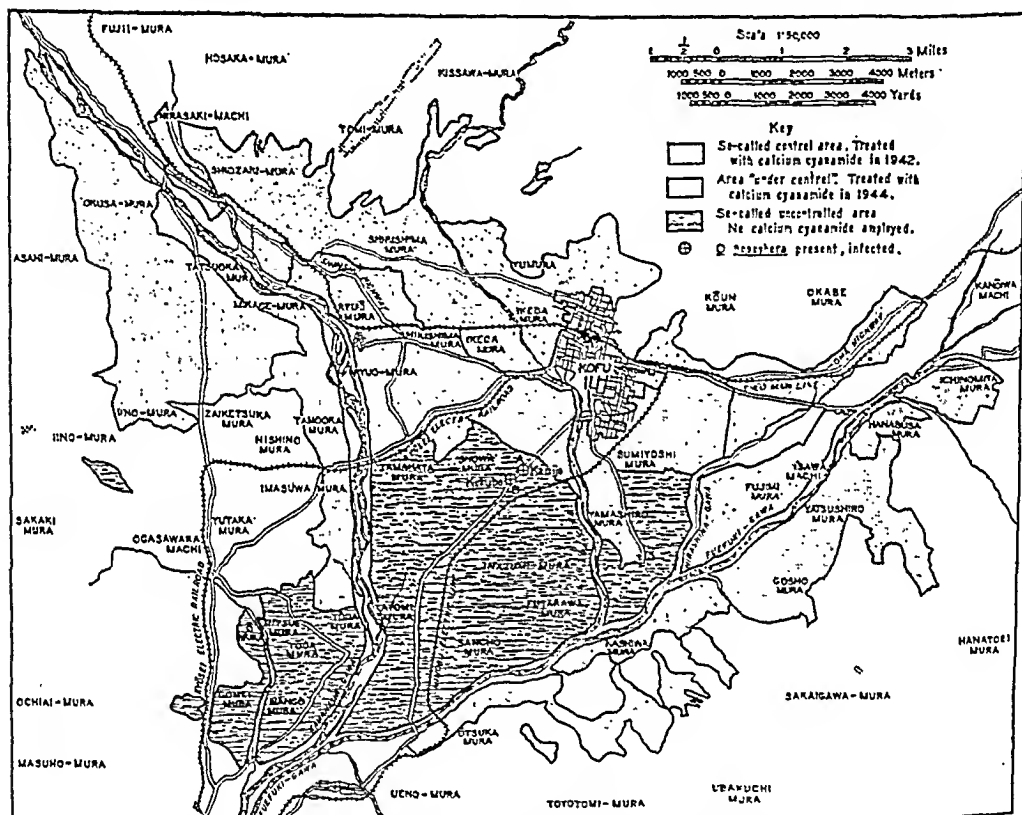


FIG. 4. THE KOFU AREA IN YAMANASHI PREFECTURE

competent microscopists for a period of 5 additional months before being permitted to continue on their own responsibility.

Most of the infection in the Kofu area is contracted through working in the rice fields. Bathing in streams in the endemic zone is prohibited by health department regulation, but nevertheless some such bathing is done. Drinking water is invariably obtained from artesian wells. Many farmers wear two pairs of trousers when working in the rice fields, and this is said to provide protection against infection. The local health authorities also recommend the wearing of rubber boots.

In 1945 the population of the endemic area was 284,987. Between May 1, 1944 and April 30, 1945, a total of 160,906 stool examinations was conducted by the control stations, of which 10,597, or 6.6 per cent, were positive for *S. japonicum*. All of these examinations were made by means of a single fecal smear with

what the commission regarded as inadequate equipment. A break-down of the examination figures is given in table 3.

In addition to stool examinations on the population in the endemic area, the prefectural health authorities have conducted stool and postmortem examinations of certain domestic and wild animals in the zone. The evidence obtained would seem to indicate that such animals are responsible to a considerable extent for the transmission of infection to the snail intermediate host. The results of these examinations are given in table 4.

TABLE 3

Results of stool examinations for schistosomiasis carried out in the Kofu area by health authorities of Yamanashi Prefecture between May 1, 1944 and April 30, 1945

AREA (Fig. 4)	NO. PERSONS EXAMINED	NO. PERSONS POSITIVE	PER CENT POSITIVE
Control.....	81,031	5,026	6.2
Under control.....	57,013	2,983	5.2
Uncontrolled.....	22,862	2,588	11.3
Totals.....	160,906	10,597	6.6

TABLE 4

Results of examinations for S. japonicum infection in lower animals in the Kofu area carried out by health authorities of Yamanashi Prefecture between May 1, 1944 and April 30, 1945

SPECIES	NO. EXAMINED	NO. POSITIVE	PER CENT POSITIVE
Cattle.....	7,059	2,184	30.9
Goats.....	1,118	158	14.1
Dogs.....	353	176	49.9
Horses.....	967	0	0
"Rats" (probably including field mice, Microtus spp.).....	1,707	656	38.4
Moles.....	68	13	19.1
Mustela sp.*.....	10	9	90.0

* Probably *Mustela (lutreola) itatsi*.

Examination of school children in Kofu area. On the basis of information furnished by the Chief of the Health Section, Yamanashi Prefecture, the commission selected four schools, two in the so-called control area and two in the uncontrolled area, for obtaining stool samples for microscopic examination. Information was obtained from the prefectural government concerning the incidence of infection in children in these schools as revealed by stool examinations between the period May 1, 1944 and April 30, 1945. This information is summarized in table 5.

Table 6 presents the results of the stool examinations by the commission on children from the same schools. It will be seen that the incidence of infection as

revealed by our examinations was more than twice that obtained in examinations in the same schools by the prefectural health department, the respective figures being 53.5 per cent and 24.2 per cent. The health department examinations included all children in the four schools, and thus comprised individuals in age groups not represented in our sampling of children between 8 and 15 years of age. The great difference in the number of examinations by the respective agencies,

TABLE 5

Incidence of schistosome infection in certain schools in the Kofu area selected by the Commission for study, based on examinations by Yamanashi Prefectural Government, 1944-1945

NAME OF SCHOOL	NO. FETILS EXAMINED	NO. POSITIVE	PER CENT POSITIVE
So-called control area			
Shiozaki.....	2,305	560	24.3
Ryuo.....	2,833	627	22.1
Uncontrolled area			
Okamada.....	1,177	267	22.7
Sancho.....	876	284	32.4
Totals.....	7,191	1,738	24.2

TABLE 6

Incidence of S. japonicum and other helminth parasites in school children (ages 8 to 15 years) examined by Commission on Schistosomiasis in Kofu area, Yamanashi Prefecture

SCHOOL	NO. EXAMINED	NO. MALES	NO. FE-MALES	NO. NEGA-TIVE	NUMBER INFECTED									
					Schistosoma japonicum				Ascaris lumbricoides		Hookworm		Trichuris tri-chiura	
					Males	Fe-males	Total	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Shiozaki	100	57	43	5	33	24	57	57.0	82	82.0	19	19.0	48	48.0
Ryuo	133	66	67	9	28	23	51	38.3	102	76.7	36	27.1	46	34.6
Okamada	106	54	52	8	44	20	64	60.4	88	83.0	34	32.1	21	19.8
Sancho	119	55	64	4	35	38	73	61.3	98	82.4	35	29.4	33	27.7
Totals	458	232	226	26	140	105	245	53.5	370	80.8	124	27.1	148	32.3

and more especially differences in sampling and ages represented in the sampling, renders impossible a comparison of results. However, for the reason that the plain sedimentation technique is much more efficient than is the fecal smear technique, it would seem that the actual incidence of infection in the children in these schools was much higher than that found in the health department surveys and in all probability more nearly approached the figure recorded in the surveys of the commission.

The incidence of intestinal helminths in the 458 children examined by the com-

mission in the Kofu area was 80.8 per cent for *A. lumbricoides*, 27.1 per cent for hookworm, and 32.3 per cent for *T. trichiura*.

Snail collections in the Kofu area. Snail collections were made in the vicinity of Kamijo and Kokubo, villages south of Kofu City. Numerous specimens of *Oncomelania nosophora*, the snail intermediate host, were found without difficulty along the margins of the irrigation ditches and in one place along the edges of the rice fields. On the return to the Tokyo laboratory of the commission, 200 snails were crushed and examined for schistosome cercariae; 3 snails were found infected.

Morbidity and mortality in the Kofu area. It was not possible to obtain records of morbidity or mortality from the prefectural health department. Dr. Saburō

TABLE 7

Age distribution of clinical schistosomiasis in 505 patients treated in the private hospital of Dr. Saburō Sugiura in the Kofu area

AGE GROUP	TOTAL NO.	NO. OF MALES	NO. OF FEMALES
1-5	23	21	2
6-10	95	85	10
11-15	48	41	7
16-20	24	11	13
21-25	34	13	21
26-30	46	18	28
31-35	29	9	20
36-40	28	15	13
41-45	43	21	22
46-50	50	23	27
50 and over	85	37	48
Totals.....	505	294	211

Sugiura stated that he treated approximately 2,000 patients a year; undoubtedly many patients are treated by other private hospitals, by the public health dispensaries, and by Yamanashi Hospital, a public institution. Likewise, little information could be obtained concerning age distribution of clinical schistosomiasis in the area. The data in table 7 were furnished by Dr. Sugiura in connection with patients treated by him in September 1944. Not much can be concluded from these data because of the relatively small number of persons involved. However, the figures indicate the preponderance of male patients over female patients; this is in keeping with a general impression that the disease is more prevalent among males because of their greater opportunities for acquiring the infection.

Chemotherapy employed in the Kofu area. Certain information was obtained concerning chemotherapeutic methods employed in the treatment of schistosomiasis in this area. Dr. Saburō Sugiura employed "Stibnal," a Japanese manufactured preparation, said to contain 0.3 per cent sodium antimony tartrate in solution. The first dose was 10 cc., the second 15 cc., followed by 20 cc. each

for a total of 20 doses, or 455 mgm. of antimony. Acute cases usually responded well to this treatment while chronic cases usually failed to show much improvement.

One of the public health dispensaries visited by the commission used "Fuadin" in the treatment of the disease, the product having been produced by the I. G. Farbenindustrie Aktiengesellschaft, Leverkusen-am-Rhine, Germany. A considerable supply of the drug was on hand. The initial dose was 1.5 cc., the second dose 3.5 cc., and the following doses 5.0 cc. each. The drug was given every other day with a total dose of 75.0 cc. This course of treatment had been employed for the past 5 years.

SURVEYS IN THE NUMAZU AREA, SHIZUOKA PREFECTURE

General description and extent of infection. The endemic area in the vicinity of Numazu (Fig. 5) covers a low-lying section comprising about 10 square miles and extending from a mile to the east of the town of Numazu westward as far as Yoshiwara. It is bounded on the north by the back road between these two towns and on the south by the main road (Tōkaidō Highway) and the railroad (Tōkaidō Main Line) which follows the shore line of Suruga Bay. On the north the area extends to the foothills of Mt. Ashitaka. The area is drained by the Numa River and its several tributaries. In the center of the area in Sudo Township (Mura) was formerly located Sudo Swamp, sometimes referred to in the literature as Lake Ukishima. However, in 1942 this swamp was drained, and the land is now devoted to rice culture. There is a general impression among local physicians and among the inhabitants of the nearby villages that the schistosomiasis morbidity rate has been materially reduced since the drainage of the swamp.

Previous data concerning the incidence of infection in the area were published in 1933 and included the results of 1,113 stool examinations made between 1920 and 1925, and 200 examinations made in 1926; of the total of 1,313 examinations, 4.0 per cent of the individuals were positive for *S. japonicum*.

Examination of school children in Numazu area. After conferences with local officials, arrangements were made to secure stool samples from a group of children attending the Sudo Township primary school. This school is located in the center of the endemic area and at the time had 1,150 pupils from the village of Sudo and the surrounding country. Table 8 gives the results of the examinations. Fourteen, or 9.0 per cent, of the 155 children examined were found infected with *S. japonicum*. A total of 143, or 92.3 per cent, were found to have *Ascaris lumbricoides*; 4.5 per cent were infected with hookworms; 22.6 per cent harbored *Trichuris trichiuria*; while *Paragonimus westermani* was found in one case. The infection rate for *S. japonicum* was over twice that encountered in the stool surveys conducted between the years 1920 and 1926, as mentioned above.

Snail collections in the Numazu area. Search for *O. nosophora* was made in irrigation ditches and rice fields in Sudo Township east and south of the village of Masukawa. No snails were found in these locations. However, numerous *O. nosophora* were located on the borders of a small swampy area (Fig. 5) on the

margin of the former Sudo swamp about $\frac{3}{4}$ of a mile east of the village of Kawashiri. This area had apparently not been drained thoroughly and was not under cultivation, although it was surrounded by cultivated land. A total of 315 snails was examined for schistosome cercariae, 100 by crushing and 215 by placing them in individual containers and observing them for the emergence of cercariae on two days with an interval of two days between observations. Of the 315 snails, 2, or 0.63 per cent, were found infected. We were informed that Mr. Y. Miyoshi, Head of the Schistosomiasis Control Office of Yamanashi Prefecture, had collected snails in the Numazu area in 1944 and had found 13 per cent infected.

Morbidity and mortality in the Numazu area. No information concerning these points could be secured from the local health officer and time was not available

TABLE 8

Incidence of S. japonicum and other helminth parasites in 166 school children (ages 11 to 13 years) examined by Commission on Schistosomiasis at Sudo in the Numazu area, Shizuoka Prefecture

PARASITE	NO. PERSONS INFECTED	PER CENT PERSONS INFECTED	MALES			FEMALES		
			Total no.	No. infected	Per cent infected	Total no.	No. infected	Per cent infected
<i>Schistosoma japonicum</i>	14	9.0	65*	12†	18.5	83*	1†	1.2
<i>Ascaris lumbricoides</i>	143	92.3						
Hookworm.....	7	4.5						
<i>Trichuris trichiura</i>	35	22.6						
<i>Enterobius vermicularis</i>	2	1.3						
<i>Paragonimus westermani</i> ...	1	0.6						
Negative.....	9	5.8						

* Sex unrecorded in 7 cases.

† Sex of one positive individual unknown.

to proceed to Shizuoka, the prefectural capital, in an effort to obtain such information from the prefectural health authorities. Dr. M. Ona of the Fuji Hospital, a private institution of Yashiwara, stated that he had been located there for 16 years and that during the past three years he had treated on an average of 5 to 6 cases each year, whereas formerly the number of patients was double these figures. He attributed the reduction of cases to the draining of the Sudo Swamp with the resulting decrease in exposure.

Chemotherapy employed in the Numazu area. Dr. Ona advised the commission that he employed "Stibnal" in the treatment of schistosomiasis, giving 10 cc. of a solution containing 0.3 per cent sodium antimony tartrate, every day for seven days and then every other day for a total of 16 to 20 doses. He claimed good results in acute cases but stated that chronic cases failed to respond satisfactorily.

SURVEYS IN THE FUKUYAMA AREA, HIROSHIMA AND OKAYAMA PREFECTURES

General description and extent of infection. The endemic area is situated immediately to the north of the city of Fukuyama and lies partly in Hiroshima and

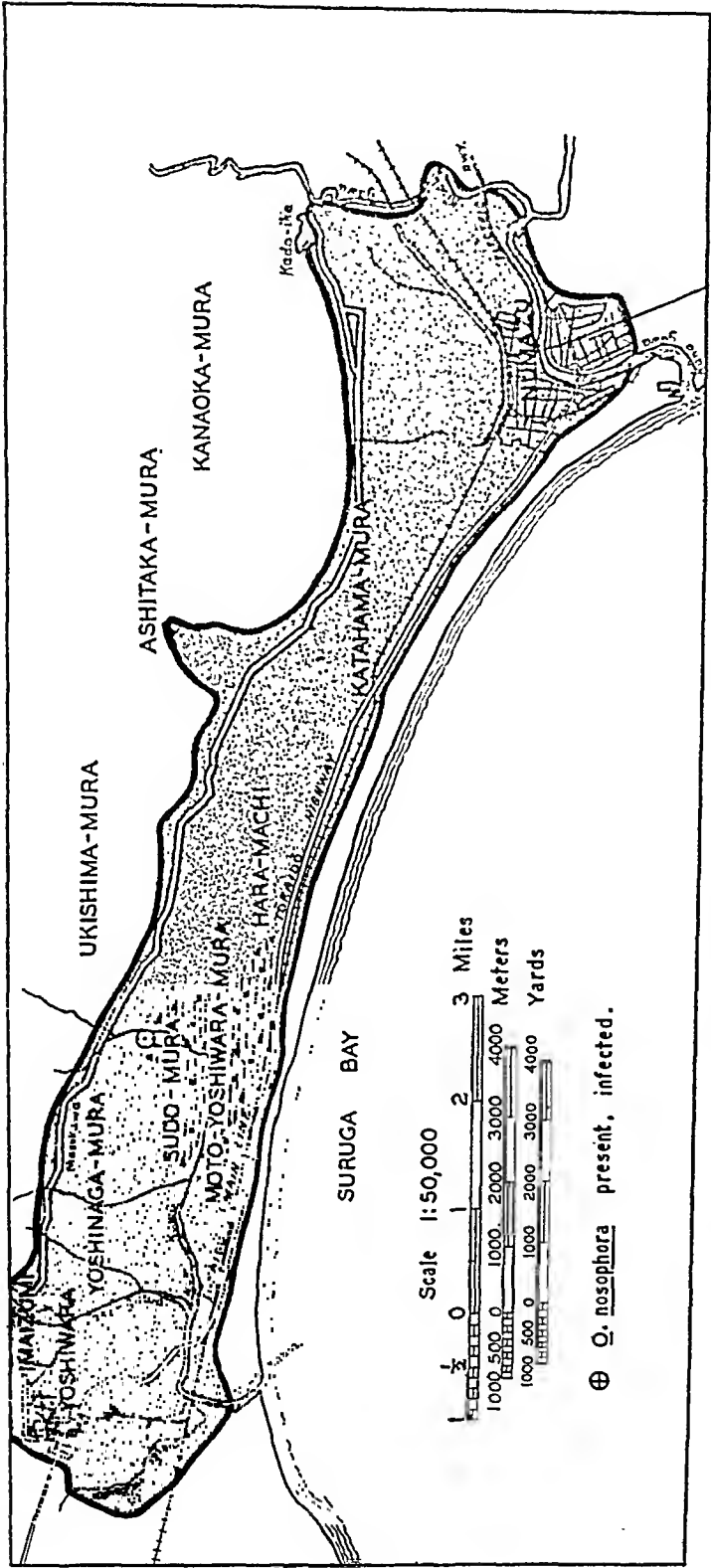


FIG. 5. ENDEMIC AREA IN THE VICINITY OF NUMAZU, SHIZUOKA PREFECTURE

partly in Okayama Prefecture (Fig. 6). It is approximately 13 miles in extent from northeast to southwest and approximately 4 miles wide at its greatest breadth. In Hiroshima Prefecture, the area includes all or part of Ekiya and Mubeyama Townships, Ashina County, and Miyuki, Michinque, Kannabe, Senda, Mino, Yuda, and Takehiro Townships, Fukayasu County, although the center of the area lies in Miyuki, Kannabe, and Mino Townships. In Okayama Prefecture, the infection occurs mainly in Takaya Township. The outline of the

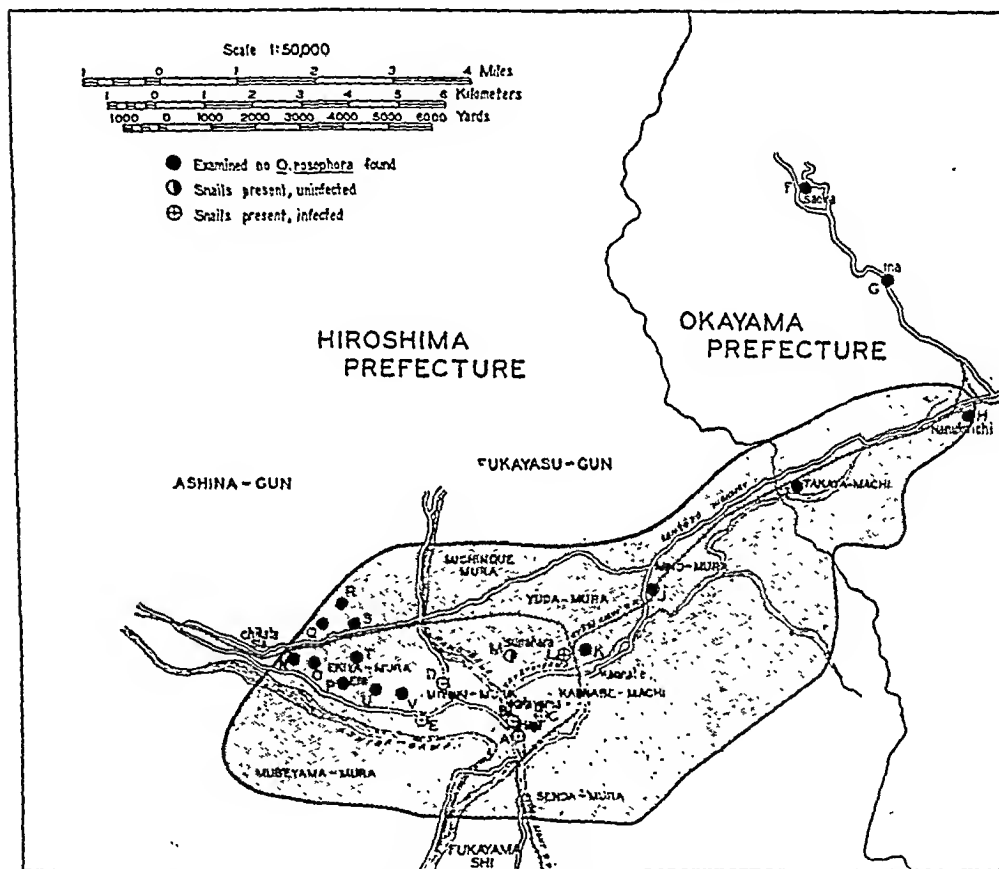


FIG. 6. ENDEMIC AREA NORTH OF FUKUYAMA IN HIROSHIMA AND OKAYAMA PREFECTURES

area on Figure 6 as the endemic zone is based on information received from many sources. The disease is probably absent from many parts of the area, but the boundaries mark the limits within which infection may exist.

During the investigations of the Commission, numerous persons were interviewed in an effort to gain information concerning the extent of infection and other phases of the problem. Much of the early research work on schistosomiasis took place in this area around the village of Katayama, from which village the disease takes its name. Investigation of schistosomiasis in this area was begun as early as 1882 when the prefectural government of Hiroshima appointed a

committee for the study of the disease (4). Organized control work was undertaken in 1918 by the "Association for the Eradication of Endemic Disease," an association which comprised 13 villages. The association had four objectives, viz: education of the population with the view of preventing acquisition of the disease; the introduction of pit privies and the holding of night soil for a period of time sufficient to allow for the destruction of schistosome eggs; the use of lime on irrigation ditches and rice fields for the destruction of the snail intermediate host; and the substitution of horses for oxen as draft animals for the reason that the former are more resistant to infection and seldom pass eggs of the parasite in the feces. Until 1938, this association supervised all control work in the area; in that year, supervision reverted to the health department of Hiroshima Prefecture. In the calendar year 1944, a total of 30,000 yen was spent in control work in Hiroshima Prefecture, one-half of this being appropriated by the prefectural government and one-half by the association of villages. Dr. Takemaro Kitajima, health officer of Hiroshima Prefecture, advised that in 1943 the program was augmented so that in addition to the lime treatment of irrigation ditches and rice fields some of the ditches were concreted, since it was found that the snails will not live in cemented ditches. However, this program was not in operation in 1944 and 1945 because of the shortage of cement.

Lime is still being used for snail destruction in parts of the area where required. The lime is applied in May at the rate of approximately 10 pounds per 5 to 10 meters of ditch, depending on the size of the ditch. This amount represents about twice that employed in the treatment of experimental plots of the commission on Leyte where it was found that other chemicals were more effective for the destruction of *Oncomelania quadrasi*, the snail intermediate host of *S. japonicum* in the Philippines. There is no question but that attempts to control schistosomiasis in this area have met with considerable success. The distribution of the snail intermediate host has been considerably restricted through the practice of liming, and the commission was unable to find snails in many areas in which they were said to have existed formerly. The educational program carried out by the "Association for the Eradication of Endemic Disease" has no doubt also contributed materially to the control of the disease. Everyone in the area is apparently quite familiar with the disease, its method of transmission, and the species of snail involved in that transmission. Even without an interpreter, it was possible to obtain from almost any man, woman, or child working in the rice fields in that area accurate information on the distribution of the snail and whether cases of the disease existed in the contiguous village. Charts showing the life cycle of the parasite and containing information in regard to the prevention of the disease were observed in some of the township schools visited by the commission.

In past years, numerous stool examinations have been made in the endemic area, and other types of surveys conducted in an effort to obtain accurate information on the distribution of infection. Effort was made to obtain information on the results of these surveys from Dr. Takemaro Kitajima, health officer, Hiroshima Prefecture, Hiroshima, Dr. T. Miyata, officer in charge Schistoso-

miasis Control Office, Hiroshima Prefecture, Fukuyama, and Mr. Shaichi Kawai, assistant to the health officer of Okayama Prefecture, Okayama. However, all records were burned during the bombing of these three cities.

Examination of school children in the Fukuyama area. After consultation with various authorities, it was decided to obtain stool samples from children in the township schools of Mino, Kannabe, and Miyuki, since these schools draw pupils from the center and from the fringes of the area in which the major portion of the disease is now confined. Table 9 presents the results of these examinations. Of the 357 individuals examined, 34, or 9.5 per cent were positive for eggs of *S. japonicum*. Percentages of infection for other intestinal helminths were 52.7 for *A. lumbricoides*, 12.3 for hookworm, 13.4 for *T. trichiura*, and 0.6 for *Enterobius vermicularis*. As in other examinations made in these surveys, the incidence

TABLE 9

Incidence of S. japonicum and other helminth parasites in school children (ages 12 to 14 years) examined by Commission on Schistosomiasis in Fukuyama area, Hiroshima Prefecture

SCHOOL	NO. EXAMINED	NO. MALES	NO. FEMALES	NO. NEGATIVE	NUMBER INFECTED											
					<i>Schistosoma japonicum</i>				<i>Ascaris lumbricoides</i>		Hookworm		<i>Trichuris trichiura</i>		<i>Enterobius vermicularis</i>	
					Males		Females		Total	Percent	No.	Percent	No.	Percent	No.	Percent
					No.	Percent	No.	Percent								
Mino-Mura	117	56	61	27	0	0	0	0	76	65.0	21	17.9	21	17.9	0	0
Kannabe	121	57	64	53	12	7	19	15.7	49	40.5	10	8.3	8	6.6	1	0.8
Miyuki-Mura....	119	60	59	39	10	5	15	12.6	63	52.9	13	10.9	19	16.0	1	0.8
Totals.....	357	173	184	119	22	12	34	9.5	188	52.7	44	12.3	48	13.4	2	0.6

figure for the latter parasite is probably far below the actual infection rate, since it is a well known fact that examination of the stool is a very inefficient method for detecting the eggs of *Enterobius*.

The incidence of schistosomiasis in the individuals surveyed was relatively low and corresponded closely with that found in the Numazu examinations. Todokoro (4) reported that 54 per cent of 104 individuals examined in December, 1909 and January, 1910 from Nakatsuhara in the endemic area in Hiroshima Prefecture were found infected with *S. japonicum*, whereas 670 examinations in this same village in 1920 revealed an incidence of 12 per cent. Unfortunately, we are unable to locate this village on any maps available to us. Kobayashi (5) in 1911 reported an incidence of infection of 54.7 per cent in 117 inhabitants of this village. In Miyuki Township, 42.3 per cent of 53 persons in the village of Moriwaki were infected, as were 30.8 per cent of 26 individuals in the village of Shimo-iwanari. In the Miyuki-Mura School, we found 12.6 of 119 children infected; this school draws pupils from the latter two villages. These data would seem to indicate that there has been a considerable reduction in the incidence of

the disease in Miyuki Township over a period of years, particularly because the technique employed in our examinations was probably much more efficient than that employed in the examinations represented in the reports of Todokoro and Kobayashi.

The failure to find cases of infection in children at the Mino-Mura School would seem to confirm the impression of health authorities that the disease is at a very low ebb in that township; this conclusion is in keeping also with the failure of the commission to find the snail intermediate host in this locality. It is apparent that at the time of the survey the disease was centered around Katayama village, an opinion which was fostered by many of the individuals who were interviewed.

Snail collections in the Fukuyama area. A thorough search for the snail intermediate host of *S. japonicum* was made throughout most of the endemic area. Collecting stations are marked alphabetically on Fig. 6. At station A near the village of Haga very few *O. nosophora* were found although specimens of *Succinia*, *Planorbula*, and *Melania* were collected. This area had been heavily limed, and a considerable amount of lime still remained in the water and along the banks of the irrigation canals. The snail was very common at collecting station B, where there was no evidence that lime had been used. Here some *O. nosophora* were found in the water of the canals and others were located in rice fields. The snails were not located in irrigation ditches or rice fields at collecting station C but were found in a small swampy area measuring approximately 5 x 100 yards; here they were located under a heavy growth of weeds and grass and appeared to be beneath the surface of the ground. Snails were also found in collecting areas D and E. Beginning above the town of Saoka at collecting area F, surveys were made south to Nanukaichi in Okayama Prefecture and west as far as collecting station J near Mino-Mura School. The area from F to G, the bridge at Ina, was covered by walking through the rice fields and examining the irrigation ditches. Here the ditches were well made, many of them of concrete, while others had concrete or rock sides with natural bottom. The water in these ditches ran swiftly and was quite clear. Very few snails of any kind were seen and no *O. nosophora* were recovered. Here the soil was more sandy and contained some gravel, in sharp contrast to the heavy black alluvial soil found near the village of Katayama. About the same conditions were encountered at collecting station H near Nanukaichi; no *O. nosophora* were found here or at collecting station I, south of Takaya, although *Melania* spp. were numerous. At collecting station J near Mino-Mura School, no *O. nosophora* were located although the snail had formerly been endemic here. At area K, north of the town of Kannabe, conditions seemed to simulate more closely those at Katayama. However, the ditches had been heavily limed and no *O. nosophora* could be found. The first snails in the valley were located at collecting station L to the northwest of the town of Kannabe. The ditches in this area had been limed also but the snails were found in small rectangular swampy areas adjacent to the irrigation canals. These swampy areas may have been abandoned fish ponds. The snails were not numerous in this area. In collecting area M south of Sunahara, a single specimen of the snail was found after a 15 minute search; the ditches in this area had been limed.

In collecting areas N, O, and P, between Chikata Station and Era, the irrigation ditches are deep with steep banks, and even in the lateral terminal canals the water flowed swiftly, a condition not favorable for *O. nosophora*; no specimens were found in these areas. Similar conditions were found to prevail in the remainder of this section at collecting stations Q, R, S, T, U, and V, and none of the snails was found in this entire region.

The information obtained from the surveys of the commission and from Japanese sources would seem to warrant the conclusion that in the Fukuyama area, *O. nosophora* is quite closely restricted at the present time to a section comprising approximately 4 square miles centering at the junction of the Kamo and Takaya Rivers. The failure of the commission to locate the snails elsewhere in the Fukuyama area does not necessarily indicate their absence; it does indicate, however, that the center of infection is in the above-mentioned area.

TABLE 10

Occurrence of cercariae of S. japonicum in Oncomelania nosophora collected by Commission on Schistosomiasis in the Fukuyama area

AREA	NO. SNAILS EXAMINED	NO. POSITIVE FOR <i>S. JAPONICUM</i>	PER CENT POSITIVE	NO. POSITIVE FOR CERCARIAE OF OTHER TREMATODES
Kannabe (Station L)	101	16	15.8*	0
Katayama (Stations A, B, and C)	200	2	1.0	0
Miyuki (Stations D and E)	200	1	0.5	0

* These snails were collected in a small marshy area in which workers in the rice fields were accustomed to defecate. Opportunities for infection were considerably greater than average.

The commission was advised by Asst. Prof. Sakae Murakami, Okayama Medical School, that in 1936 or 1937 he crushed 300 to 400 snails collected in the area between Katayama and Kannabe and found about 3 per cent infected with *S. japonicum*. He stated that at the present time the snail is found in Okayama Prefecture only in the vicinity of Takaya.

Table 10 gives the results of snail examinations by the commission for cercariae of *S. japonicum* in specimens collected in the Fukuyama area.

Morbidity and mortality in the Fukuyama area. No information could be obtained on these points for Okayama Prefecture. Mr. Kawai, representative of the health department of that prefecture, stated that all records of his office had been burned in the bombing of the city of Okayama and he did not know whether there were any clinical cases of the disease at the present time.

All records of morbidity and mortality of the disease in Hiroshima Prefecture had also been destroyed in the burning of the city of Fukuyama. However, Dr. Miyata, in charge of the control office at Fukuyama, furnished the fragmentary data listed in table 11.

It was learned that the above-mentioned figures are not based on cases occurring in the private practice of physicians in the area but represent cases diagnosed

on physical examination by teams of physicians going from village to village within the endemic area.

Mr. Ryoichi Sato, a veterinarian and president of Miyuki-Mura, stated that it is the general impression that individuals acquire a certain resistance to schistosomiasis through early exposure to the disease. During the war when lime was difficult to obtain, control work in his township had to be neglected. As a result, the number of snails increased, and there were a good many clinical cases of the disease in individuals in the teen ages because they were said to have escaped infection at an early age. Mr. Sato told the Commission that during the war many men of military age from his township were rejected for military service because they were suffering from schistosomiasis.

TABLE 11

Morbidity and mortality from schistosomiasis in Hiroshima Prefecture, 1941-1945

YEAR	NO. CLINICAL CASES	NO. DEATHS
1941	226	9
1942	About 200	2
1943	1,472	No information
1944	401	No information
1945	No information	No information

SURVEYS IN THE KURUME AREA, SAGA AND FUKUOKA
PREFECTURES, ISLAND OF KYUSHU

At Kurume, conferences were held with Mr. Monzo Uno, officer in charge, health department, Kurume City, and with Dr. T. Inoue who conducted the Inoue Hospital, a private institution. A visit was made also to the Kyushu Medical School at Kurume and various members of the faculty interviewed. At Saga, Mr. H. Yokota, Chief of Foreign Affairs, and Dr. S. Sumita, Chief of the Sanitary Section, Saga Prefecture, supplied valuable information.

General description and extent of infection. The endemic area of schistosomiasis centered around Kurume in general follows the course of the Chikugo River (Fig. 7). The area extends approximately 12 miles from east to west and approximately 9 miles from north to south at its greatest length and breadth; it covers approximately 100 square miles and lies within the county of Mii, Fukuoka Prefecture, and the county of Miyaki, Saga Prefecture.

No sustained program of control has been carried out in the area, although we were informed that lime was tried at one time without avail. The Chief of the Sanitary Section, Saga Prefecture, stated that before the war he had inaugurated a program of sanitary privy construction aimed at the control of schistosomiasis but that this work had been interrupted. He had devised a type of privy involving the use of three separate septic tanks in successive compartments so that the fecal material would be carried over from one compartment to another before being used as night soil. He had found by experimental work that the time required for the passage of the fecal material through the three compartments

permitted the natural destruction of schistosome and other helminth ova. This official recommends that human feces be permitted to stand in open containers for six months before being placed on the fields. However, he stated that few

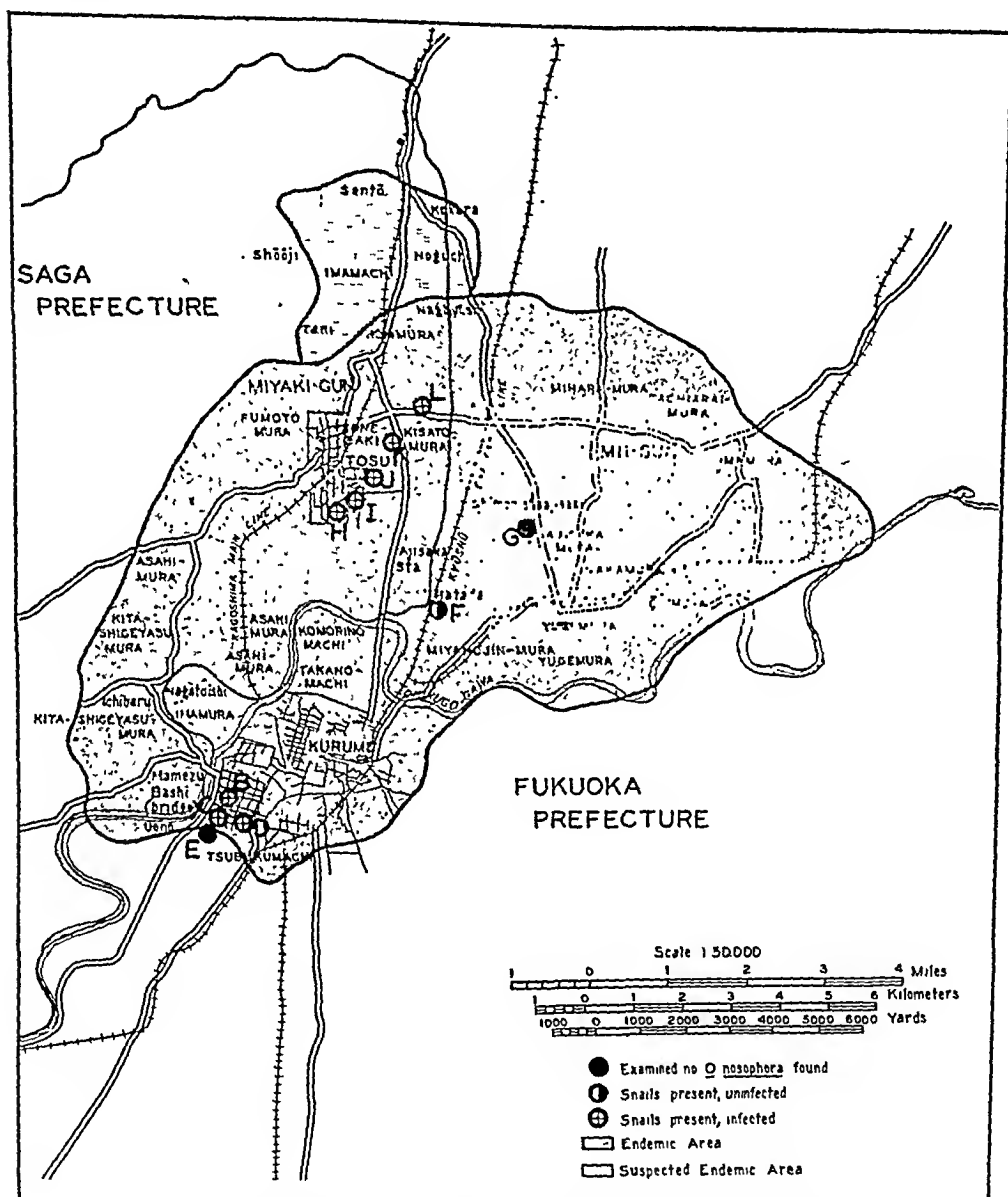


FIG. 7. THE KURUME AREA IN SAGA AND FUKUOKA PREFECTURES, ISLAND OF KYUSHU

farmers follow his recommendations and that little has been accomplished from such a measure in the way of control.

Dr. Inoue has conducted a considerable amount of research work on schistosomiasis and was able to give the commission valuable information. He also

accompanied the commission into the field for the collection of the snail intermediate host. In one of his papers, Dr. Inoue (6) cited certain figures for surveys for schistosomiasis carried out in this endemic area. These data were translated and appear in table 12.

Further information was obtained concerning infection in the endemic area from another paper by Dr. Inoue (7) and is presented in table 13. The data in table 14 were furnished by the Chief of the Sanitary Section, Saga Prefecture, and represent results of surveys conducted by prefectural health authorities during 1943 and 1944. Certain information was obtained also from the Prefectural Health Officer, Fukuoka Prefecture; the data appeared in the annual report of that official for 1942 and are given in table 15.

The information furnished by the results of stool examinations in this endemic area over the past 20 years would seem to indicate that the distribution of the

TABLE 12

Results of skin tests and stool examinations by Dr. T. Inoue in the endemic area of schistosomiasis around Kurume. (1940)

TOWN	SKIN REACTIONS			STOOL EXAMINATIONS OF SKIN TEST POSITIVES*							
	No. tested	No. positive	Per cent positive	<i>S. japonicum</i> positive		<i>Ascaris lumbricoides</i> positive		Hookworm positive		<i>Trichuris trichiura</i> positive	
				No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Nagatoishi.....	125	74	59.2	50	67.6	14	18.9	4	5.4	13	17.6
Asahi.....	975	330	33.8	29	8.8	190	57.6	75	22.7	76	23.0
Minamishigeyasu.....	471	128	27.2	5	3.9	55	43.0	11	8.6	25	19.5
Kyōmachi.....	379	46	12.1	1	2.2	3	6.5	0	0	2	4.3
Torihai School.....	822	151	18.4	15	9.9	37	24.5	9	6.0	11	7.3

* Single fecal smear.

disease is spotty in nature in that some areas have a high incidence rate and other areas a low incidence rate. The data indicate further that there has been little apparent reduction in the incidence of infection in the area over the period in question.

Examination of school children in the Kurume area. After consultation with various authorities and a consideration of the available data, three schools were selected from which to obtain stool samples from the pupils. These schools were as follows: Nagatoishi and Ajisaka, Fukuoka Prefecture, and Tosu, Saga Prefecture. Information concerning the incidence of past infection was available from previous surveys and the schools were distributed throughout the heart of the endemic area. The results of the examinations are given in table 16.

Of 114 pupils in the Nagatoishi School, 49, or 43 per cent, were found to be infected with *S. japonicum*. In the Ajisaka School, 19.3 per cent of 119 individuals harbored the infection, while 13.7 per cent of 95 pupils in the Tosu School had eggs of the parasite. Insofar as could be learned, all previous surveys in the Kurume area were based on a single fecal smear. The difference in techniques

and differences in numbers examined make it difficult to draw any comparison between the results obtained by the commission and those obtained in previous surveys. However, it is evident that there has been little, if any, decrease in the

TABLE 13

Results of stool examinations for schistosomiasis in the endemic area in Saga and Fukuoka Prefectures. (Data from paper by Dr. T. Inoue.)

TOWN	DATE OF SURVEY	NO. EXAMINED	NO. POSITIVE	PER CENT POSITIVE
Kiyama.....	1921	276	2	0.7
Fumoto.....	1921	180	4	2.2
Kizato.....	1921	185	47	25.4
Tosu.....	1921	333	116	34.8
Asahi.....	1921	147	81	55.1
Kitashigeyazu.....	1921	301	35	11.6
Minamishigeyasu.....	1921	120	9	7.5
Nagatoishi.....	1919	776	455	58.6
Nagatoishi.....	1924	80	46	57.5
Nagatoishi.....	1924	56	22	39.3
Nagatoishi.....	1927	693	332	47.9
Nagatoishi.....	1933	115	38	33.0
Nagatoishi.....	1934	115	49	42.6
Nagatoishi.....	1934	1,062	140	13.2
		2,897	1,082	37.3
Miyanozin.....	1919	158	53	33.5
Miyanozin.....	1924	142	24	16.9
Miyanozin.....	1931	623	152	24.4
Yamakawa.....	1930	179	1	0.6
Yamakawa.....	1931	1,805	1	0.1
Ogoyi School.....	1925	74	10	13.5
Yuge School.....	1930	177	14	7.9
Ogi School.....	1930	238	1	0.4
Kushiwara.....	1930	128	20	15.6
Modo.....	1935	620	125	20.2
Ajisaka.....	1921	513	296	57.7
Fukuda.....	1934	1,375	24	1.7
Fukuda.....	1935	2,152	36	1.7

schistosomiasis infection rate in the areas in question over the period represented by the several surveys.

The per cent of infection with intestinal helminths in the 328 children examined in the Kurume area was 59.5 for *Ascaris lumbricoides*, 5.8 for hookworm, 6.7 for *Trichuris trichiura*, and 0.6 for *Enterobius vermicularis*.

Snail collections in the Kurume area. At collecting station A (Fig. 7) immediately to the southeast of Ichibaru near the Chikugo River, numerous specimens

of *O. nosophora* were found in the irrigation ditches and in one area the snails were encountered in abundance around the stems of rice plants previously har-

TABLE 14

Results of stool surveys for schistosomiasis conducted by Health Department of Saga Prefecture, 1943-44

TOWN	DATE OF SURVEY	NO. EXAMINED	NO. POSITIVE	PER CENT POSITIVE
Kizato	1943	360	62	17.2
Kizato	1944	370	62	16.8
Tosu*	1943	474	89	18.8
Tosu*	1944	474	89	18.8
Asaki	1943	619	117	18.9
Asaki	1944	1,481	235	15.9
Kitashigeyazu*	1943	420	19	4.5
Kitashigeyazu*	1944	420	19	4.5

* There appears to be some repetition of figures in these data in that in two towns the same number of individuals with the same number of positives is recorded for both 1943 and 1944. However, the interpreter insisted that the data are correct.

TABLE 15

Stool examinations for schistosomiasis conducted by Health Department of Fukuoka Prefecture, 1942

TOWN	DATE OF SURVEY	NO. EXAMINED	NO. POSITIVE	PER CENT POSITIVE
Kurume City	1942	244	32	13.1
Yamanato	1942	1,926	2	0.1

TABLE 16

Incidence of *S. japonicum* and other helminth parasites in school children (ages 8 to 13 years) examined by Commission on Schistosomiasis in Kurume area, Saga and Fukuoka Prefectures

SAGA AND FUKUOKA PRELECTURES																
SCHOOL	NO. EXAMINED	NO. MALES	NO. FE-MALES	NO. NEGA-TIVE	NUMBER INFECTED											
					<i>Schistosoma japonicum</i>				<i>Ascaris lumbricoides</i>		Hookworm		<i>Trichuris trichiura</i>		<i>Enterobius vermicularis</i>	
					Males	Fe-males	Total	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
Nagatoishi....	114	60	54	30	32	17	49	43.0	48	42.1	3	2.6	4	3.5	2	1.8
Ajisaka.....	119	59	60	24	17	6	23	19.3	81	68.1	10	8.4	13	10.9	0	0
Tosu.....	95	49	46	21	9	4	13	13.7	66	69.5	6	6.3	5	5.3	0	0
Totals.....	328	168	160	75	58	27	85	25.9	195	59.5	19	5.8	22	6.7	2	0.6

vested. It was apparent that the irrigation canals had flooded and washed the snails into the fields, since the stems of the plants had been bent over by the rush of the water. Snails were also found in abundance at collecting stations B, C, and D, south and east of the Mamezu bridge and not far from the banks of the

river: The snails were numerous in the small irrigation canals even though the area was well drained and most of the ditches dry; about 1,000 specimens were collected in this area. At station E near Ueno no *O. nosophora* were found.

Collections at station F near Hatada consisted of only a few specimens; this area is south of Ajisaka school. At station G south of Shimo-nishiajisaka, no *O. nosophora* could be found. Very few snails were located in an area south and east of Tosu (collecting stations H, I, J), although search was made at numerous places beginning 1.5 miles south of the town and working around the eastern outskirts. Likewise, specimens were not numerous at collecting station K north and

TABLE 17

Results of examination for schistosome cercariae of *O. nosophora* collected by the Commission on Schistosomiasis in the Kurume area

AREA	NO. SNAILS EXAMINED	NO. WITH MATURE S. JAPONICUM	NO. WITH IMMATURE FORMS	PER CENT INFECTED	NO. WITH CERCAIRAE OF OTHER SPECIES OF TREMATODES
Ichibaru (Station A)....	100	7	0	7.0	0
Mamezu Bridge (Stations B, C, D).....	200	4	4	4.0	1
Tosu-Sonezaki (Stations H, I, J, K and L).....	210	16	4	9.5	9

TABLE 18

Results of examinations of snails in the Kurume area for cercariae of *S. japonicum* by Matsubara *et al.* (1942)

AREA	NO. COLLECTING STATIONS IN AREA	PER CENT S. JAPONICUM INFECTION IN SNAILS
Mamezu Bridge.....	2	0 to 7.3
Nagatoishi.....	6	0 to 30.0
Miyaki County.....	8	0 to 2.17
Fukuoka Prefecture.....	9	0 to 6.45

east of Tosu. However, east of the village of Sonezaki at station L about 800 *O. nosophora* were collected in an irrigation ditch which was entirely dry. Table 17 gives the results of the examination of samplings of snails for schistosome cercariae.

To supplement the information given in table 17 on the infection rate of *S. japonicum* in snails collected by the commission, certain data were obtained from the paper by Matsubara *et al.* (8). The data have been consolidated by districts as indicated in Table 18. In the experience of the commission, infection rates in snails such as those cited in Table 17 and 18 indicate a marked incidence of schistosomiasis in the population. It is believed also that such rates indicate that there has been little reduction within recent years of the schistosomiasis incidence rate in this area.

Morbidity and mortality in the Kurume area. The Chief of the Sanitary Section, Saga Prefecture, advised that there had been reported in that prefecture an average of 395 cases of clinical schistosomiasis per year during the past 5 years. The schistosomiasis morbidity rate in Saga Prefecture has been approximately 134 per 10,000 population. Similar data were not available for Fukuoka Prefecture, but would probably not vary widely from the Saga Prefecture figures, since part of Fukuoka Prefecture contains some highly endemic foci of the disease.

No information was available concerning the mortality from the disease. Dr. Inoue stated that he treated about 80 cases of the disease per year in his hospital; the age of the patients usually ran between 8 and 18 years. He had no definite information concerning the number of deaths traceable to schistosomiasis, but believed that there are very few.

Chemotherapy employed in the Kurume area. Dr. Inoue advised that he employed "Nesbosan" in the treatment of schistosomiasis. This is a p-aminophenylstibinate derivative containing in each ampoule 0.05 gram of antimony and manufactured by Banyu Pharmaceutical Co., Ltd., Honcho, Nihonbashi, Tokyo. He used 1 ampoule every 3d day for a total dose of 0.1 to 0.15 gram. Good results are said to be obtained in acute cases.

SUMMARY

The five known endemic areas of schistosomiasis in Japan were surveyed by the Commission on Schistosomiasis with the view of ascertaining the extent of these areas and gathering information which would be pertinent to the prevention of this disease in military personnel. The methods employed in the surveys included the compiling of all available information from national, prefectural, and local health authorities, the collection and examination for schistosome eggs of stool samples from children in certain representative schools in each area, and the collection and examination of *Oncomelania nosophora*, the snail intermediate host of *Schistosoma japonicum*, in an effort to determine its distribution within the area and the rate of infection with cercariae of the parasite.

The endemic areas surveyed were as follows:

- (1) The Tone River area in Chiba and Ibaraki Prefectures.
- (2) The Kofu area in Yamanashi Prefecture.
- (3) The Numazu area in Shizuoka Prefecture.
- (4) The Fukuyama area in Hiroshima and Okayama Prefectures.
- (5) The Kurume area in Saga and Fukuoka Prefectures, island of Kyushu.

Individuals found infected with *S. japonicum* on stool examination have been reported from other areas including Tochigi, Aomori, and Fukui Prefectures. National health authorities were of the opinion that such cases do not represent infection acquired in these Prefectures but rather imported infections. A relatively large number of such cases has been reported from Fukui Prefecture (1) but it was the view of Japanese health officials and parasitologists that these reports are either in error or represent cases of infection acquired elsewhere. In reply to a specific inquiry, the Health Officer of Fukui Prefecture stated that there were

no cases of the disease in that Prefecture. Schistosomiasis was formerly endemic in certain parts of Tokyo Prefecture, but it is said that all these foci of infection have now been eradicated.

The Tone River area, which lies partly in Chiba Prefecture and partly in Ibaraki Prefecture, apparently has the lowest infection rate of any area in Japan. While the disease is still endemic in certain parts of this river valley, it is at a very low ebb. An examination by the commission of 390 school children in six localities revealed only 3 cases of the disease. The commission was unable to find specimens of *O. nosophora* in any place in which a search was made, although the snail undoubtedly still occurs in certain sections. However, the entire river valley east of the junction of the Kinu River should be regarded as a possible endemic area.

From the standpoint of incidence of infection and morbidity rate, the Kofu area, Yamanashi Prefecture, is the most important one in Japan. Of 458 children from four schools in which examinations were conducted by the commission, 245, or 53.5 per cent, were found infected. In spite of the fact that a control campaign has been aggressively carried on in this area since 1942, it is apparent that little has actually been accomplished in the reduction of the incidence of infection or the number of clinical cases.

The Numazu area in Shizuoka Prefecture covers only about 10 square miles and is the smallest focus of the disease in Japan. Only 9 per cent of 155 children from a school in the center of the area were found infected. Authorities stated that there has been a marked decrease in the number of clinical cases of schistosomiasis in this area since the drainage of a large swamp in the heart of the area in 1942.

The Fukuyama area in Hiroshima and Okayama Prefectures ranks third in importance of the endemic areas in Japan. Examination of 357 school children in the area indicated an infection rate of 9.5 per cent. Through the organized control work which has been carried on in this area over the past 30 years, the disease has been gradually reduced and the distribution of the snail intermediate host considerably restricted. At the time of the present surveys, the infection seemed to be confined principally to the townships of Kannabe and Miyuki in Fukuyasu County of Hiroshima Prefecture, with the center of the focus near the village of Katayama where the disease was first discovered.

The Kurume area in Saga and Fukuoka Prefectures, island of Kyushu, is approximately of the same size as is the endemic focus at Kofu, Yamanashi Prefecture. However, the infection is more spotty than in the Kofu area, some sections showing a high incidence of the infection while in other sections the disease is at a much lower ebb. Examination of 328 children in three schools within the area revealed an infection rate of 25.9 per cent. The distribution of the snail intermediate host within the area varies considerably, the snails being found in abundance in the high incidence zones and few in number or absent in certain other sections where the incidence was found to be lower. This situation is apparently due to the operation of natural factors since practically no control work has been carried out in this area.

A total of 1,688 school children between the ages of 8 and 15 years was examined by the commission in the five endemic areas. Of these, 381, or 22.6, were infected with *S. japonicum*. Due to a confusion in records, the sex of 14 individuals was not recorded; one of these individuals was positive. Of the remainder, 802 were males, of whom 234, or 29.2 per cent were infected, while 872 were females, of whom 146, or 16.7 per cent, were found positive. This considerable difference between the infection rates in males and females confirms the impression of Japanese health authorities and physicians that males more frequently suffer from the disease due probably to their greater opportunities for acquiring infection.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to Brigadier General Guy B. Denit, M.C., U.S.A., Chief Surgeon, United States Army Forces, Pacific, for the opportunity of carrying out the surveys in question and for the encouragement which he lent to the work of the commission during its tour of duty in the Southwest Pacific and in Japan. We are greatly indebted also to Brigadier General George W. Rice, M.C., U.S.A., Surgeon, Eighth Army, and Brigadier General Wm. M. Hagins, M. C., U.S.A., Surgeon, Sixth Army, for the facilities which they placed at the disposal of the commission and for their very cordial cooperation. Colonel A. H. Schwichtenberg, M.C., U.S.A., and members of the staff of the Advance Echelon, Chief Surgeon's Office, were particularly helpful in arranging many of the details connected with the work and their cooperation is greatly appreciated. The commission is indebted to Colonel Thomas Wornham, U.S.M.C., Commanding Officer, 27th Marine Regiment, 5th Marine Division, for the courtesies and facilities extended in the way of quarters, rations, and transportation during its stay in the Kurume area. Lt. Col. Donn J. Robertson, U.S.M.C., Executive Officer, 1st Lieut. Leslie F. Fultz, U.S.M.C., S-1, Capt. Francis J. Lutz, U.S.M.C., S-4, Lieut. Commander Thomas E. McGeachy, M.C., U.S.N.R., Regimental Surgeon, Lieut. (jg) George Levy, M.C., U.S.N.R., and Lieut. (jg) A. Littman, M.C., U.S.N.R., rendered valuable assistance to the work of the commission, while 1st Lieut. Richard Woodard, U.S.M.C., and Pfc. Dewitt Trowbridge, U.S.M.C., are to be thanked for their service as interpreters. Miss Sumiko Nakano also served as interpreter during the surveys in the Kurume area.

During the work in the Fukuyama area, the commission was quartered with the 3d Battalion, 162d Infantry, 41st Infantry Division; appreciation is accorded Captain John E. Norton, Inf., A.U.S., Battalion Commander, for his many courtesies. Miss Emiko Fujii acted as interpreter during the commission's stay at Fukuyama.

The commission should like to acknowledge also the considerable assistance which it received from Dr. Nobutaro Ishii, Chief, Research Laboratory of Parasitology, Government Institute for Infectious Diseases, and his assistant, Miss Yasuko Mitoma, both of whom accompanied the commission during the surveys in the Tone River, Kofu, and Numazu areas.

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BLOCK RESIDUAL SPRAYING OF PREMISES WITH DDT FOR THE CONTROL OF MALARIA

E. HAROLD HINMAN AND LAWRENCE K. CUTKOMP¹

The technique of DDT residual spraying has presented an entirely new approach to the control of malaria. Comparatively large scale tests have been made with this insecticide, both in the United States and in other countries. The Tennessee Valley Authority (see Metcalf *et al.*, Hess and Keener) has been interested in the development of this technique and its utilization as a routine malaria control measure. The present study was initiated to determine the efficacy of block spraying of all structures on premises with residual DDT for malaria control.

One of the first field trials in this country was reported by Gahan and Lindquist (1945) carried out at Stuttgart, Arkansas, in one of the largest rice growing sections in the United States. The inside walls and ceilings of nearly all buildings, as well as the bridges and culverts, in two areas of 9 square miles each were sprayed with DDT with an average deposit of 56 mgm. per sq. ft. in one area and 208 mgm. of DDT in the other. Chicken houses, pump houses, and nail kegs were selected as checking stations. These authors found a 99 per cent reduction of adult *Anopheles quadrimaculatus* in the heavily treated area and 91 per cent reduction in the lightly treated areas as compared to adjacent untreated zones. Sampling of the larval population in the two treated areas compared to larval abundance in untreated areas showed a 63 per cent reduction in the heavily treated area and 57 per cent in the lightly treated one.

Trapido (1946) reports upon the effect of residual spraying of dwellings with DDT in the control of malaria transmission in Panama with special reference to *A. albimanus* as carried out in a highly malarious area which has been under continuous study for 15 years and where conditions are excellent for the continuous production of large numbers of the malaria vector. In this experiment the exterior and interior of all dwellings (native huts) were sprayed. This test of the efficacy of DDT residual spraying for malaria control is considered to be more severe since *A. albimanus* does not normally rest in houses during the daytime. This study revealed that not only was there a large reduction in numbers of mosquitoes in treated dwellings, but among those taken in these dwellings there was a marked reduction in the per cent engorged (since DDT activates the insect and it loses interest in feeding). Also, among the engorged mosquitoes the 24-hour survival rate was low for a period of 3 months after treatment. A marked decline in the malaria rate was observed, the cumulative index being 14.8 per cent for the year 1945 in the sprayed village and 52 per cent for the same one-year period at the control villages.

Hess and Keener (1947) conducted tests in the Tennessee Valley during

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the summer of 1945 utilizing the precipitin test of blooded *A. quadrimaculatus* collected from premises before and after DDT residual spraying to determine the percentage which had fed on humans. They found that prior to treatment the average percentage of human blood meals for house-caught mosquitoes (5.5 per cent) was approximately twice that for the barn-caught mosquitoes (2.7 per cent). Of the total adults collected from premises an average of 15 per cent was found in the houses, 79 per cent in the barns, and 6 per cent in other structures. The residual spraying effected an almost complete elimination of mosquitoes from the houses during the remainder of the season and approximately 25 per cent reduction in human-fed mosquitoes resting in barns adjacent to treated houses immediately after treatment. The total immediate reduction in the human-fed mosquito population of the entire premise was approximately 50 per cent as a result of the house spraying. Therefore, residual house spraying alone in areas of serious potential hazard of malaria transmission might not provide sufficient protection, and the treatment of entire premises might greatly increase the effectiveness of the operation. The precipitin data also indicated that house mosquito-proofing alone, even if it were 100 per cent effective, would probably reduce by more than 50 per cent the total number of human blood meals taken by *A. quadrimaculatus* on the average premise studied. It was suggested by these authors that premise spraying with DDT may be more effective in malaria control than house mosquito-proofing.

Gahan and Payne (1945) carried out a study of the effectiveness of DDT residual spray in a rice growing area in Mexico where *A. pseudopunctipennis* is the vector. Not only was there a marked reduction (99.9 per cent) in the number of adults in the treated area but also a considerable reduction (89 per cent) in the larvae in the breeding grounds immediately adjacent to the treated region as compared with untreated control villages.

EXPERIMENTAL AREAS AND TECHNIQUES

While the interest of the TVA is primarily in the control of malaria associated with its reservoirs, this organization consistently has been interested in integrating its program with the efforts of those responsible for the control of anophelines arising from sources outside but adjacent to the reservoirs. Several years ago in an attempt to increase the effectiveness of the over-all control program, the TVA mosquito-proofed residences in certain areas under special circumstances, within one mile of the reservoirs, as a temporary measure with the collaboration of the State and local health Departments.

As part of the DDT experimental program during the summer of 1945 a series of 3,150 houses within one mile of the Kentucky, Wheeler, and Gunter'sville Reservoirs were treated with DDT residual spray. In view of the promising results obtained and also the findings of Hess and Keener, referred to above, it was decided to carry out a wide scale experiment in the Wheeler Reservoir during 1946 utilizing the DDT residual spray technique inside all structures of the premises.

The experimental "treatment" zone includes what is known as the Barren

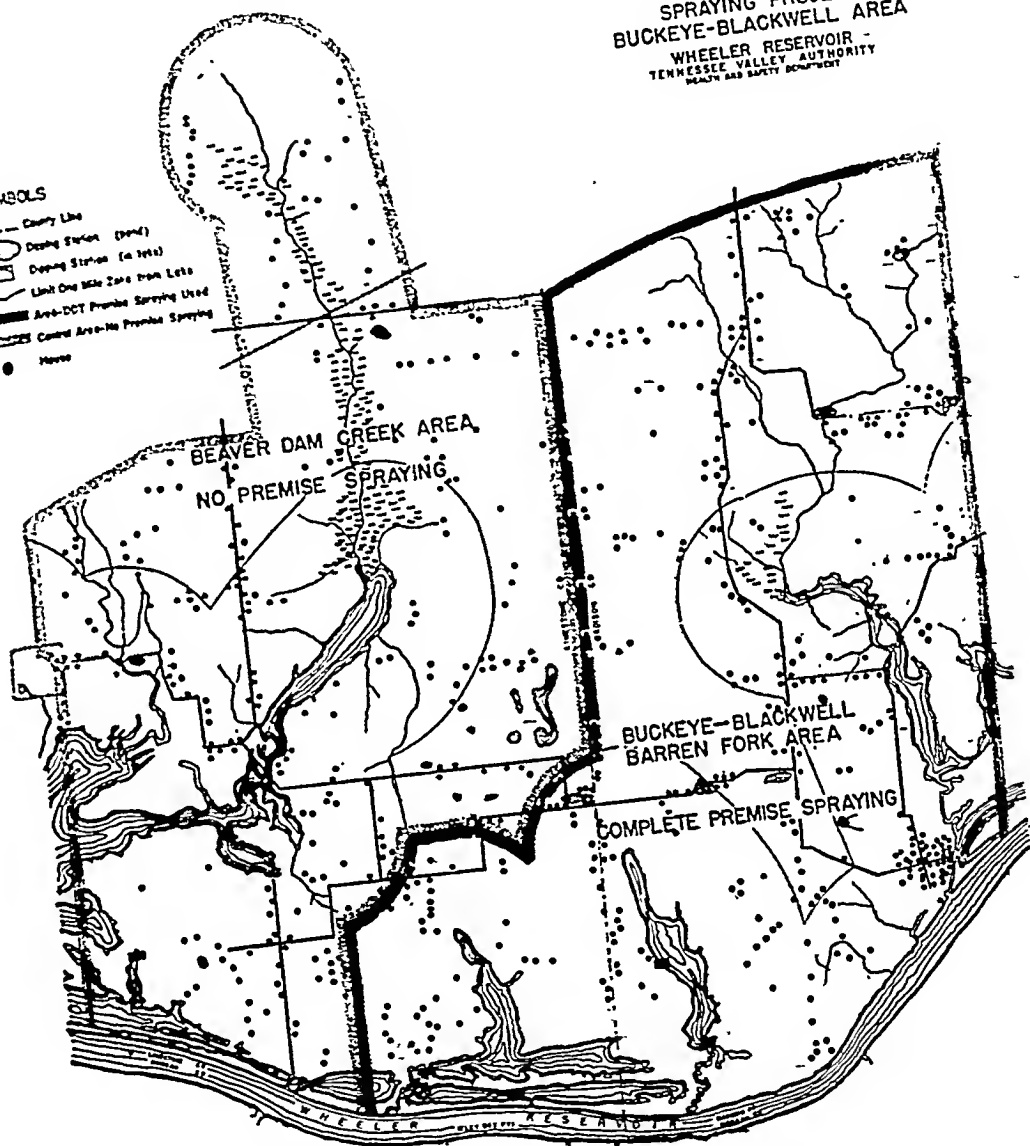
BLOCK RESIDUAL SPRAYING OF PREMISES WITH DDT

Fork-Blackwell Swamp-Buckeye area of Wheeler Reservoir in North Alabama, together with adjacent territory outside the one-mile zone. See Map 1. A

MOSQUITO CONTROL
COMPLETE PREMISE
SPRAYING PROJECT
BUCKEYE-BLACKWELL AREA
WHEELER RESERVOIR
TENNESSEE VALLEY AUTHORITY
HEALTH AND SAFETY DEPARTMENT

SYMBOLS

- County Line
- Dipping Station (Dond)
- Dipping Station (in 1942)
- Unit One Mile Zone from Lake
- ▨ Area-DDT Premise Spraying Used
- ▤ Control Area-No Premise Spraying
- House



MAP 1

total of 299 occupied premises were treated in an area of 38.1 square miles. This area has been under more or less constant intensive observation for the past 8 years in connection with studies on mosquito-proofing. Actually only a portion of it has been mosquito proofed. It is recognized as one of the two zones of highest mosquito production within the Wheeler Reservoir. The

Blackwell and Buckeye sections were set up for diking and dewatering several years ago, but the advent of the war prevented the acquisition of the necessary pumping equipment. In view of the anticipated elimination of these areas from the reservoir, no attempt has been made to control aquatic vegetation in the area, and for this reason the anopheline potential is much greater than it would be otherwise. Furthermore, there are many extensive *A. quadrimaculatus* natural breeding places throughout the area outside the reservoir.

The Beaver Dam-Limestone Creek area (See Map 1) which has served as a control during the season for investigations reported is not strictly comparable to the treated zone. Within this portion of the reservoir stands of aquatic vegetation are not so extensive as in the treated area but, as will be shown later, *A. quadrimaculatus* occurred in considerable numbers. The control area under study includes 32.7 square miles with 238 occupied premises. No DDT or other insecticidal material was applied by the TVA in the area. Approximately the same percentage of mosquito-proofed residences occur in the two areas.

During the period June 7-28 a 5 per cent emulsion of DDT (stock solution 25 per cent DDT, 73 per cent xylene, and 2 per cent Triton) was applied to all structures in the Barren Fork-Blackwell-Buckeye area which would be expected to afford resting places for *A. quadrimaculatus* mosquitoes. The application was by means of E-Z knapsack sprayers equipped with flat-spray nozzles, No. 1/4T-8002 (manufactured by Spraying Systems Co., Chicago, Illinois). A tabulation of the structures treated follows:

TYPES OF STRUCTURES TREATED

STRUCTURES	NUMBER TREATED
Houses.....	295
Porches.....	349
Barns.....	237
Privies.....	199
Pig pens.....	186
Chicken houses.....	249
Churches.....	2
Stores.....	2
Miscellaneous.....	115
Total.....	1,634

The spray was applied at the rate of approximately 200 mgm. of DDT per sq. ft. to the inside walls, ceilings, screens, except in barns where an arbitrary height of approximately 10 feet was selected and no spraying carried out above this level. Where houses were elevated above ground, the accessible portion of the under surface was sprayed. Mattresses were sprayed if the occupants of the houses desired. This work was done in collaboration with the Alabama State Health Department and the Madison County Health Department. In order to promote cooperation, mimeographed sheets from the respective County Health Departments were distributed to the occupants of both areas, explaining

the purposes of the study. Only two refusals were encountered. Three barns which have been utilized as regular adult mosquito checking stations were left untreated.

MOSQUITO DENSITY RESULTS

In North Alabama the *A. quadrimaculatus* breeding season was somewhat retarded in the spring of 1946, but by early June significant densities of this species occurred. Owing to delays in obtaining personnel, it was not possible to initiate the checking of premises for mosquito density and abundance of other insects until approximately the first of June. Furthermore, it was necessary to concentrate first on the inspection of premises to be sprayed. Therefore, the pretreatment inspection of the area sprayed slightly antedated the collection of similar data from the control area. Prior to the application of the spray, all the structures on every premise were inspected and counts of anoph-

TABLE 1
Average number of *A. quadrimaculatus* mosquitoes per premise

AREA	PRE- TREAT- MENT INSPEC- TION	POST-TREATMENT INSPECTIONS								
		July			August			September		
		#1	#2	#3	#4	#5	#6	#7	#8	#9
Sprayed.....	20.00	0.4	0.4	0.9	1.1	1.2	1.2	2.1	2.8	6.2
Control.....	23.3	32.9	32.1	35.6	43.8	31.1	13.1	15.1	29.4	14.2

elines recorded, together with information on the abundance of flies, roaches, bedbugs, et cetera. Following the spraying, a group of 75 representative premises throughout each of the areas were selected for regular inspection. In the selection of these, the premises with the highest numbers of anophelines found on the pretreatment inspection were chosen but selected to provide adequate geographic coverage of the areas. Following the application of the spray each selected premise was inspected once every 10 days (3 times monthly), and on the second round in the month of August sufficient personnel was available to make a complete inspection of every building on each of the 537 premises. Occasionally individual owners or tenants were absent and their premises locked at the time of the inspection, and for this reason there is a slight variation in the number of premises utilized in obtaining the averages reported in table 1.

Table 1 is based upon the average of the total number of *A. quadrimaculatus* counted in all structures of a premise. It is noted that prior to treatment approximately similar numbers of adult *A. quadrimaculatus* occurred in both the sprayed and unsprayed areas, but that in the treated area after application of DDT a very marked decline in the number of mosquitoes occurred and persisted throughout the balance of the season. When the total numbers of anoph-

elines within the houses only are compared (table 2), differences between sprayed and unsprayed areas are found to occur, even though the numbers are small.

The small number of anophelines encountered in occupied houses as compared to barns was notable even upon the first inspection prior to any spraying and was very striking in both areas and irrespective of whether houses were screened or unscreened. It is in contrast to the findings of Hess and Keener in the Kentucky Reservoir where an average of 43 *A. quadrimaculatus* were encountered per house, although undoubtedly higher anophelism occurred in the Kentucky Reservoir. Hewitt and Kotcher (1941) found from 6.3 to 8.0 mosquitoes per house as an average on three weekly inspections of 15 unscreened houses in the Buckeye area during the months of July and August 1940. Records from barns as checking stations indicate that the 1940 anopheline density in the Barren Fork, Blackwell Swamp, Buckeye, Beaver Dam, and Limestone Creek areas of Wheeler Reservoir was approximately twice as great as in the 1946 season. It

TABLE 2
Average number of *A. quadrimaculatus* mosquitoes per house

AREA	PRE-TREATMENT INSPECTION	POST-TREATMENT INSPECTIONS								
		July			August			September		
	June	#1	#2	#3	#4	#5	#6	#7	#8	#9
Sprayed.....	1.07	0.00	0.01	0.00	0.00	0.01	0.04	0.01	0.01	0.09
Control.....	0.40	0.50	0.19	0.45	0.19	0.20	0.17	0.36	1.39	0.13

is possible that our low counts may be associated with the application by the occupants of insecticidal repellents and sprays. The abundance of new furniture within these houses makes it obvious that there has been an improvement in economic status, possibly permitting the tenants to purchase more sprays than in past years. Other unknown factors may have been responsible for the low counts within houses.

Both tables 1 and 2 indicate that DDT premise residual spray is effective in reducing the numbers of *A. quadrimaculatus* resting in these premises. Further evidence of the abundance of the anopheline in the area was obtained in two distinct methods. One barn, premise No. 15, was sprayed in one half only. The counts for the 10 successive inspections in the unsprayed half were as shown in table 3.

During this entire period no count in excess of 6 anophelines was obtained in the sprayed half after the treatment had been applied. These results are very similar to those reported by Metcalf et al (1945).

In addition, a number of nail kegs were maintained close to the reservoir and the natural breeding places in both experimental and control areas. While many of these were frequently tampered with or actually removed by the public, a number were not molested and continued to give evidence of high anopheline

density throughout the entire season with some showing counts consistently above 100 in the sprayed area.

Table 4 summarizes the catches of *A. quadrimaculatus* from a wooden nail

TABLE 3
Counts of A. quadrimaculatus in unsprayed half of a barn

INSPECTION	NUMBER OF <i>A. QUADRIMACULATUS</i> MOSQUITOES
Pretreatment	600
Post-treatment:	
1	95
2	240
3	301
4	348
5	613
6	245
7	457
8	885
9	439

TABLE 4
Keg records of adult A. quadrimaculatus—east shore of Blackwell Swamp

DATE	MALES	FEMALES	TOTAL
May 17	1	10	11
30	7	9	16
July 9	9	21	30
11	45	52	97 (2 kegs)
18	79	86	165+ (2 kegs; some mosquitoes not recovered)
29	157	37	194
August 1	117	80	197
12	377	274	651
20	282	103	385
26	604	430	1,034 (2 kegs)
September 11	66	20	86+ (Some not recovered)
25	81	31	112

keg located immediately adjacent to Blackwell Swamp (treated area). The predominance of males indicates the proximity to breeding place and recency of emergence. This table indicates the high production which occurred in the area throughout the season, despite the premise spraying. Mosquitoes were collected from one keg except where noted. When collected from two kegs, those in one keg exceeded those in the other keg because of the selectivity of the mosquitoes.

LARVAL DENSITY

In order to obtain an index of anopheline population in the two areas and particularly to ascertain if the spraying of all structures in a substantial area would have a significant effect upon anopheline breeding, regular dipping studies were carried out both within the reservoir and in natural breeding places outside of it in both the control and treated areas. Sampling was performed utilizing the "square foot" dipping technique (Hess, 1941). Breeding conditions in the two areas within the reservoir, Blackwell Swamp (in sprayed zone) and Lower Beaver Dam Creek (in control zone) are comparable, and vegetation was predominantly lotus. The natural breeding areas (ponds) outside the reservoir were quite heterogenous and contained a wide variety of vegetation, including

TABLE 5

Comparison of larval density in sprayed and unsprayed areas—Wheeler Reservoir, 1946

AREA	NUMBER OF LARVAE PER SQUARE FOOT													
	Date													
	6-21	6-28	7-5	7-12	7-19	7-26	8-2	8-9	8-16	8-23	8-30	9-6	9-12	9-20
Sprayed														
Blackwell Swamp.....	0.4	0.5		0.7	2.3	0.8	2.1	1.0	2.1	1.2	0.7	1.4	0.6	1.1
Betts Spring.....		4.5	1.2	0.8	0.4	1.2	5.1	4.2	1.9	0.3			6.3	10.2
Barren Fork.....		2.1		1.8	0.8	1.2	0.5	0.9	0.2	1.1	1.5	1.4	0.3	1.2
Swancott C.....		1.3	1.1	0.8	1.3	0.8	0.1	0.5	0.5				0.4	0.3
Swancott B.....		1.3	1.6	1.7	3.0									
Control														
Lower Beaver Dam.....		0.3				0.9	0.5	0.8	1.0	0.9	0.6	1.2	1.0	0.6
Limestone.....	3.8		0.5	0.1	0.1	0.5	1.2	1.8	0.6	0.5	1.3	2.0	2.4	7.0
Railroad Pond.....		1.7	0.8	0.4	0.7	2.1	0.3	1.0	2.5	2.9	2.0	1.1	0.4	

smartweeds (*Persicaria*), lizard tail (*Saururus cernuus*), spike rush (*Eleocharis*), *Isnardia*, water primrose (*Jussiaea grandiflora*), and various sedges.

There was considerable variation in water level in the natural breeding grounds in accordance with rainfall, and this may have exerted an influence on the density of *A. quadrimaculatus* larvae. Table 5 summarizes the density of larvae from plots within the sprayed area including the Blackwell Swamp portion of Wheeler Reservoir and four ponds outside the lake in the treated area. One of these, known as Swancott Pond B, went dry in the middle of July and had water at only irregular intervals thereafter. Table 5 gives similar data from Beaver Dam plot within the reservoir and two ponds outside the lake—the three plots occurring in the unsprayed zone. The tabulated data indicate the average number of *A. quadrimaculatus* larvae per square foot based upon 20 square-foot samples.

In addition, data were available from routine larvae dipping by inspectors

in both the treated and untreated areas. This information is summarized in table 6.

It will be noted that wide variations in larval density occurred in practically all of these sampling stations. There are no consistent trends in either the stations from the treated or untreated areas, and there are no consistent differences between the sprayed and unsprayed areas. From these data it would be concluded that premise residual spraying with DDT in a large block had no measurable effect on anopheline population under the conditions of this study.

TABLE 6
Anopheline larval density—Wheeler Reservoir, 1946

DATE	SPRAYED AREA			CONTROL AREA		
	Number of Dips	Number of Larvae	Number of Larvae Per 100 Dips	Number of Dips	Number of Larvae	Number of Larvae Per 100 Dips
5-3	100	0	0.00	250	12	4.80
5-10	485	21	4.33	445	3	0.67
5-24	195	42	21.54	140	6	4.29
6-7	650	39	6.00	250	19	7.60
6-14	85	28	32.94	90	25	27.78
6-28	250	23	9.20	120	10	8.33
7-12	690	94	13.62	220	29	13.18
7-19	905	132	14.59	150	5	3.33
7-26	330	7	2.12			
8-2	620	484	78.06	180	11	6.11
8-9	440	322	73.18	70	0	0.00
8-16	580	638	110.00	100	0	0.00
9-13	140	24	17.14	60	47	78.33

MALARIA SURVEYS

During the last two weeks of September 1946, in cooperation with the Limestone and Madison County Health Departments² a house-to-house thick film malaria survey was undertaken. In the sprayed area 1,116 slides were obtained, and in the unsprayed area 830 films were made. It is estimated that slides were taken from nearly 90 per cent of the inhabitants of these two zones. Examination of these films revealed no positives in either group.

In the fall of 1945 a malaria survey of the inhabitants living within one mile of the reservoir gave the following results:

In the Barren Fork-Blackwell-Buckeye (treated) area from 594 slides 2 positives were found, while among 345 slides from Beaver Dam-Limestone (control) area no positives were found.

It is recognized that during a period of such low endemicity it is not possible to draw any conclusions with reference to the influence of DDT residual spraying on the transmission of malaria.

² Dr. Arthur M. Shelamer, Health Officer.

COST OF DDT RESIDUAL SPRAYING OF PREMISES

Records maintained of the cost of premise spraying including transportation, materials, laborers, and foreman, but not supervision, indicated that the average cost per premise was \$3.92 (based upon cost of \$1.55 per gallon for 25 per cent DDT, 75 cents per hour for laborers, \$1.13 per hour for foreman, and 10 cents per mile for operation of truck).

On an average an estimated total of 4,324 sq. ft. of area were sprayed per premise. Of this an average of 2,854 sq. ft. were residential, and 1,470 sq. ft. were barns and other outbuildings. It might, therefore, be assumed that the cost of residual spraying all structures as compared to the house alone is only

TABLE 7
Occurrence of Flies Within Houses

INSPECTION	PERCENTAGE OF HOUSES WITH INDICATED NUMBER OF FLIES					
	Less than 10 flies		From 10 to 50 flies		More than 50 flies	
	Sprayed	Control	Sprayed	Control	Sprayed	Control
Pretreatment	5	0	23	62	72	38
Post-treatment:						
1) July	89	0	11	75	0	25
2) July	92	4	4	55	4	41
3) July	95	24	5	56	0	20
4) August	94	33	6	28	0	39
5) August	81	23	18	54	1	23
6) August	91.6	56	7.8	38	0.6	6
7) September	93	56	7	42	0	2
8) September	81	37	19	57	0	6
9) September	73	27	27	68	0	5

increased about 50 per cent under rural situations comparable to those encountered in this area. In the Wheeler Reservoir during 1945 a comparable estimated cost for spraying houses alone was \$3.00.

EFFECT OF RESIDUAL SPRAY ON INSECTS OTHER THAN MOSQUITOES

Owing to limitations of personnel it was not possible to obtain as accurate information as was desired concerning density of houseflies, flies about the stables, cockroaches, bedbugs, et cetera in these premises. However, inspectors were instructed to maintain records of approximate abundance as, for example, less than 10, from 10 to 50, or more than 50, particularly in the case of flies in dwellings. Also, statements were requested from the occupants of these premises as to annoyance from flies and other household or barn inhabiting insects.

Table 7 summarizes counts of flies in the houses of the two areas under study. The percentage of houses where flies were either counted or estimated and re-

corded as less than 10, from 10 to 50, or as having more than 50 flies is listed. It may be seen from a study of this table that prior to treatment the great majority of houses (72 per cent) had more than 50 flies per house, whereas for three months after the spraying practically no houses had as many as 50 flies and very few as many as 10. In the control area the majority of houses throughout the entire study had from 10 to 50 flies per house per inspection.

The occurrence of significant numbers of flies in the sprayed premises is attributed to two factors: First, the longer time necessary for killing flies (possibly due to their great activity and very brief period of contact with the sprayed surface and to their frequent alighting on unsprayed surfaces); and secondly, to the fact that they are active during the daytime and, therefore, flies recently entering the house and still surviving are in evidence. The large number of dead flies visible speaks for the efficacy of residual spray.

The householders in the treated areas who requested that mattresses be sprayed owing to the occurrence of bedbugs indicated greatly increased comfort and absence of these pests following the treatment. Similarly, many of the individuals called attention to absence of roaches, chicken mites, and fleas.

DDT RESIDUAL SPRAY IN A MALARIA CONTROL PROGRAM

In consideration of the habits of *A. quadrimaculatus*, particularly its period of activity, which tend to bring it into contact with man about the premises, its preference for daytime resting places, its frequent alternating visits from the breeding grounds to premises in search of blood or ovipositing situations, together with the long interval between the obtaining of the infective blood meal and its ability to transmit, it is believed that DDT premise residual spraying will exert a very effective barrier between man and the mosquito. Thus, the repeated exposure to treated surfaces would appear to be an effective barrier against the transmission of malaria. The extension of the spraying to all structures of the premise should increase the efficacy of the operation as compared to spraying of the house alone.

As referred to above, the precipitin studies by Hess and Keener indicated that mosquito-proofing alone could not reduce by more than 50 per cent the total number of human blood meals taken by *A. quadrimaculatus* under the conditions of our study. It would, therefore, appear that premise residual spraying may be more effective than mosquito-proofing in preventing malaria transmission. On the basis of these studies, the TVA is recommending the extension of DDT residual premise spraying in lieu of maintenance of mosquito-proofing for the special conditions in which this secondary measure has been utilized in its malaria control program, and no expansion of mosquito-proofing is anticipated at present. The annual cost of mosquito-proofing maintenance is approximately three times the cost of DDT residual premise spraying under the conditions occurring in this area.

It is desired to stress the importance not only of residual treatment of all structures on a premise, but also to emphasize that the measure should be employed upon a "block" basis. Two reasons are involved in this if our objective

is to prevent the transmission of malaria. In the first place it is necessary to eliminate any protected places where anophelines may freely come and go without exposure to DDT. In the second place intermingling of inhabitants from the sprayed premises to the non-sprayed or vice versa may enhance the opportunity first for infection of the anopheline and subsequently for the transmission of the parasite.

POTENTIAL COLLATERAL BENEFITS OF DDT RESIDUAL SPRAY

The present study provided further evidence of the utility of DDT in the control of houseflies and other household pests. The public health significance of these side effects is obvious.

DURATION OF EFFECTIVENESS OF DDT RESIDUAL SPRAY

From an epidemiological point of view, it is believed that a spraying applied early in June would provide protection against malaria transmission for a whole season (latter part of September) under the conditions of this experiment. In latitudes closer to the equator, it is probable that additional applications may be necessary.

SUMMARY

1. All structures of 299 premises in a block of over 38 square miles in Wheeler Reservoir, North Alabama, were sprayed in June with approximately 200 mgm. of DDT per sq. ft., and a similar block was maintained as a control. Inspections of more than one-fourth of all premises in the two areas were made three times each month during July, August, and September and densities of *A. quadrimaculatus* recorded. The single application of spray markedly reduced the number of mosquitoes for a period in excess of three months. It appears from our observation that residual spraying of premises provides a more effective barrier to the transmission of malaria than does house spraying alone.

2. The study did not elicit any significant decline in mosquito density in the treated area as indicated by larval density, counts from keg shelters or the untreated surfaces of barns.

3. The added cost in this study of spraying all structures on premises was not in excess of one-third the cost of spraying the houses alone—\$3.92 compared to \$3.00.

4. The importance of treatment of large blocks of premises is emphasized.

5. On the basis of previous information on human blood feeding of *A. quadrimaculatus* and the present observation of almost complete elimination of this species within the premise by DDT residual spraying of all structures, it appears that complete DDT residual spraying offers more protection against malaria transmission than mosquito-proofing with its practical limitations.

6. Many beneficial side effects as regards a public health program result from the residual spray application including the control of other disease transmitting and pest insects.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the collaboration of the Alabama State Health Department and the Madison and Limestone County Health Departments in the planning and execution of this study. A number of representatives of the Health and Safety Department of the Tennessee Valley Authority rendered assistance in the program. Mr. C. D. Fairer, field sanitary engineer, Wheeler Reservoir, was most helpful in the actual spraying operation; Mr. George Keener, Jr., assisted in the sampling for larvae; Messrs. W. Marshall, James H. Sellers, and W. O. McGee participated in the inspection for adult mosquitoes; Mr. C. C. Kiker and Dr. A. D. Hess have provided helpful advice throughout the investigation.

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OBSERVATIONS ON THE LABORATORY CARE, LIFE
CYCLE, AND HOSTS OF THE CHICKEN MITE,
DERMANYSSUS GALLINAE^{1,2}

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INTRODUCTION

Evidence has been accumulating during the past few years to indicate that domestic fowl may play an important part in the epidemiology of certain neurotropic viruses. Since the viruses of St. Louis encephalitis and Western equine encephalomyelitis have been isolated recently from the chicken mite in nature (Smith, Blattner, Heys, 1944; Sulkin, 1945), it seemed advisable to investigate experimentally the possible role of this arthropod in the dissemination of equine encephalomyelitis.* It is the purpose of this paper (1) to review the life cycle of the chicken mite, *Dermanyssus gallinae*, (2) to describe methods which have been found successful in the collection, storage, feeding, and manipulation of the mite, and (3) to discuss the possible hosts.

MATERIALS AND METHODS

Source of mites: Originally, mites were obtained from farms in the vicinity of Dallas, Texas. However, it soon became apparent that a much richer source lay within the city limits where inexperienced individuals stimulated by shortages attempted to raise chickens in crowded quarters with no program of parasite control.

Methods of manipulating and feeding mites in the laboratory: Collection and transfer of the mites was accomplished by means of a suction device as shown in Plate I, Fig. 2. Such an arrangement of tubes confined the mites to small containers which were easily detached, closed, and stored (Plate I, Fig. 3). For most purposes very fine meshed bolting silk was used to close the bottom end of the storage tubes since it retained all stages of the mite. The circles of silk were cut with a cork borer of appropriate size and were fastened to the ends of the tubes with Duco Household Cement.

In the laboratory vacuum was obtained from an ordinary filter pump type of aspirator. In the field the same equipment was used except that suction was applied by mouth.

Mites for immediate use and observation were kept in the storage vials in a chamber at room temperature and a relative humidity of approximately 70 per

¹ For the morphology and systematic classification of *D. gallinae* the reader is referred to EWING (1922, 1929), and Banks (1915).

² This work was supported by a grant from the Rose Lampert Graff Foundation, Los Angeles, California.

*Reeves and his associates (Science 105: 411-412, 1947) have recently reported recovery of the Western equine virus from wild bird mites (*Liponyssus sylviarum*).

cent. Otherwise, they were stored in the refrigerator at 4-5°C. At this latter temperature the mites became inactive, a useful fact because they can be removed from the tubes in this inactive state by merely inverting and tapping gently. To prevent excessive condensation of moisture on the mites in the refrigerator the small tubes were sealed in rubber-stoppered 1 x 8 in. test tubes.

Feeding was routinely performed on young chickens placed in a Fernbach culture flask. The mouth was then covered with bolting silk (Plate I, Fig. 1). A small raised platform inside the flask aided in preventing the chicken from crushing the fragile engorged mites. It was often necessary to direct the air current from a small electric fan into the mouth of the flask to prevent condensation of moisture from the chicken for even a light film would cause the death of many mites. As the excreta from the chickens would also drown many mites, the following methods were used to overcome this difficulty:

(1) A small flattened wad of cotton soaked in collodion was placed over the anus and perianal region of the chicken and allowed to dry in place.

(2) A purse-string suture was placed about the anal orifice. This was drawn tight after a small wad of cotton had been placed in the cloaca. A final daub of collodion finished the procedure. This method was the most reliable, but it had the disadvantage of being permanent.

(3) An ordinary rubber condom cut down to about one-third length was stripped over the caudal end of the bird after the feathers had been trimmed from that region. A seal of isobutyl methacrylate polymer dissolved in xylene was satisfactory. This method gave excellent results. It could be removed without difficulty, thus allowing the chicken to survive.

When mites were fed on animals other than chickens, containers of a size which would provide the animal sufficient room and yet keep the mites in close contact were employed. In the case of man, a small chamber cut from a homeopathic vial leaving only the neck and 1 cm. of the body was taped to the skin. Thus, mites could be introduced, left for any given period of time, and then could be removed readily by means of the suction device described above.

It was found necessary to cover the top of all containers used in feeding with fine meshed cloth since the mites would crawl out and destroy themselves in any moat of oil or water. Moistening the cloth cover daily with a small amount of dimethyl phthalate solution on the end of a pipe cleaner discouraged the collection of mites about the mouth of the flask.

Mite colony: A small chicken coop was built on the medical college grounds. This structure included a screened run and a roosting box with a hinged roof. Numerous wooden slats were lightly nailed about the inside of the box. Chickens were maintained in the coop. Mites introduced into the roosting box multiplied rapidly and could be found easily by lifting the slats with a knife blade. This colony provided a constant source of mites throughout the summer and fall. A similar coop built indoors would allow propagation during the winter months.

For studies in which it was necessary to observe closely and confine all stages, the mites were stored in the tubes and fed by the methods already described.

Storage of mites: When it was necessary to store large numbers of mites for long periods of time the storage tubes were sealed in rubber-stoppered 1 x 8 in. test tubes and were placed in the refrigerator at 4-5°C. It was found possible to keep mites readily available throughout the year in this manner, especially if they were fed at intervals of three to four weeks followed by a few days at room temperature to allow oviposition and hatching of ova. Many survived after four months storage in such containers with no feedings and with no attempt to control humidity.

LIFE CYCLE

Wood (1917) of the Bureau of Entomology presented an accurate account of the life cycle of *Dermanyssus gallinae* which closely coincides with our observations. By strange coincidence he chose Dallas, Texas, as the site of his studies because of the prevalence of the mite in this region. It will suffice to mention briefly the various phases in the life cycle as illustrated in Plate II.

Adult female mites usually begin oviposition in from 12 to 24 hours after feeding and lay as many as 7 eggs which hatch into larvae in 48 to 72 hours at summer temperatures. The sluggish six-legged larva molts without feeding into the eight-legged active first-stage nymph in 24 to 48 hours. After a blood meal molting into the second stage nymph takes place in 24 to 48 hours. A short rest period may follow but usually another feeding takes place promptly, followed by molting into the adult form. Wood (1917) reported occasional instances of a third nymphal stage.

The life cycle is very rapid and has been reported by Bishopp and Wood (1917) to take place in as short a period as 7 days. Under natural conditions feeding is accomplished mainly at night, the mites leaving the fowls and secreting themselves in cracks during the day.

DISTRIBUTION

It is said that the chicken mite is probably distributed throughout the entire world where chickens are raised (Ewing, 1922). It is found throughout the United States though it is more prevalent in the Southwest (Bishopp and Wood, 1917). Gibson (1930) states that it is widely distributed throughout both Eastern and Western Canada.

OTHER HOSTS

The literature available to us concerning hosts other than chickens, either natural or experimental, has been rather limited. Table 1 lists the hosts reported by various authors. With the exception of Man and certain unqualified references to rats and mice, the hosts reported for the mites have been birds.

Table 2 lists the results of the trials on animals which have been tested experimentally in our laboratory as hosts for the chicken mite. It will be noted that the mites fed on all of the birds and on none of the mammals tested. In each case starved mites were kept in close contact with the test animal for a

period of hours. It was noted that mites refusing to feed on certain animals would engorge readily on chickens immediately after removal from that animal.

In the case of white mice, animals of diverse ages were tested. These included one hairless mouse three days old, one mouse two and one-half weeks old, and

TABLE 1
Animals upon which D. gallinae has been reported to feed

HOST	AUTHOR
Aves	
"Cage birds"	Banks (1915)
Turkeys	Wood (1917)
Pigeons	Bishopp and Wood (1917)
Swallows	Patton (1931)
"Sparrows"	Patton (1931)
English sparrows	Ewing (1922)
Canaries	Ewing (1922)
Towhee	Ewing (1922)
Mammalia	
Rats	Patton (1931), Banks (1915), Boyt (1937), Arnold and Arnold (1943), Lawrence (1935), Ciliento (1923)*
Mice	
Man	

* Some of these authors describe the mites as "attacking" man.

TABLE 2
Animals experimentally tested as hosts for D. gallinae in this laboratory

ANIMAL	NO. TESTED	RESULTS
Aves		
Chickens	15	Feeding took place readily in all animals tested
Pigeons	4	Feeding took place readily in all animals tested
English sparrows	2	Feeding took place readily in all animals tested
Mammalia		
White swiss mice	4	None fed
White rats	2	None fed
Man	5	None fed

two adult mice. The wild mice were adult specimens trapped on the premises. Five different human subjects, two males and three females, were tested.

From time to time reports of chicken and bird mites attacking man have appeared in the medical literature (Arnold and Arnold, 1943; Boyt, 1937; Lawrence, 1935; Ciliento, 1923). Arnold and Arnold (1943) described the lesions produced as grouped urticarial papules with no visible punctum occurring

on the parts of the body exposed during sleep. The source of the mites was frequently found to be in deserted bird nests near bedroom windows. In only one instance (Boyt, 1937) has the affected individual or his associates actually noted engorgement of the mites. This information was obtained second-hand by the author. Our findings concur with those of Wood (1917) in that though mites crawling on the skin produce a disagreeable sensation, none have ever been observed to engorge or bite. This does not exclude lesions produced by products of the mites on sensitive individuals.

All attempts to induce mites to feed upon freshly-drawn chicken blood *in vitro* were uniformly unsuccessful.

SUMMARY

1. Methods for collecting, feeding, storing, and manipulating the mite, *Dermanyssus gallinae*, have been discussed.
2. The life cycle is reviewed with the aid of photomicrographs.
3. Experimental data on the ability of a variety of animals to serve as hosts for the chicken mite is presented.
4. The question of these mites attacking man is discussed.

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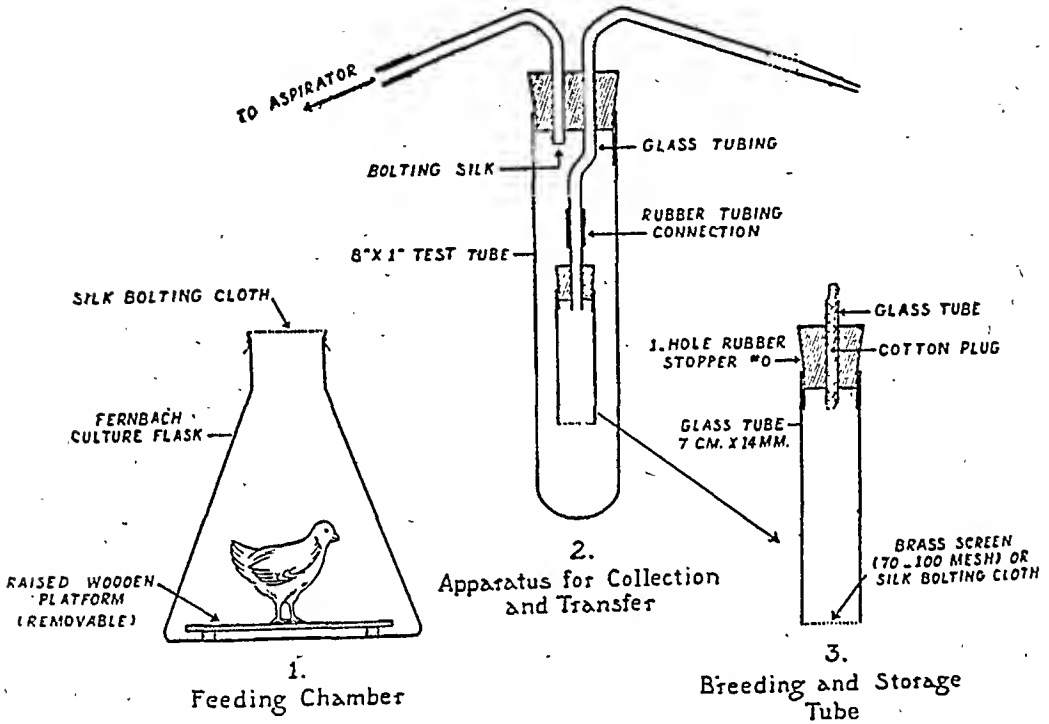


PLATE I

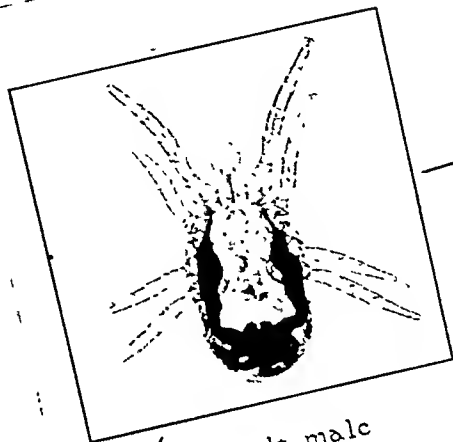
APPARATUS FOR THE COLLECTION, FEEDING, AND STORAGE OF THE CHICKEN MITE

FIG. 1. Feeding chamber. A Fernbach culture flask containing a small wooden platform (assembled in sections) will admit a small chicken. The mouth is covered with a layer of bolting silk to prevent the escape of the mites.

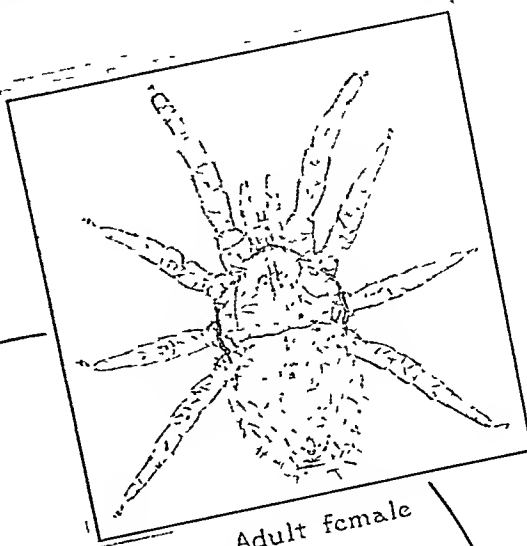
FIG. 2. Apparatus for the collection and storage of chicken mites.

FIG. 3. Breeding and storage tube. The silk bolting cloth was found to be the most practical covering for the bottom of the tube. One or two such tubes may be sealed in a 1 x 8 inch test tube for storage in the refrigerator.

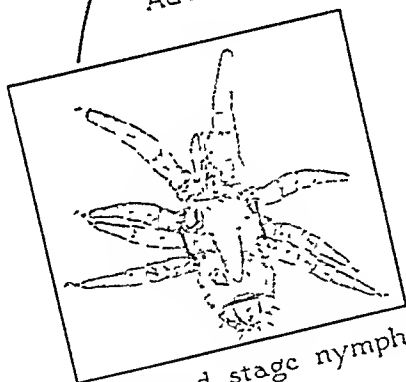
OBSERVATIONS ON THE CHICKEN MITE



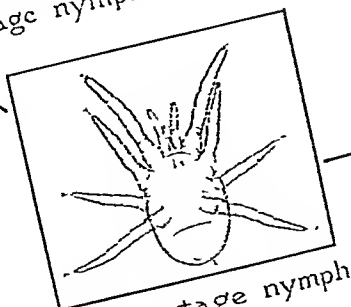
Adult male



Adult female



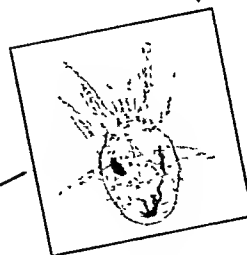
Second stage nymph



First stage nymph



Ovum



Larva

PLATE II
 LIFE CYCLE OF THE CHICKEN MITE, *Dermanyssus gallinae*
 All figures to scale. (Photomicrographs by Medical Art Department, Southwestern
 Medical College.)

STUDIES ON THE CYCLIC PASSAGE OF YELLOW FEVER VIRUS IN SOUTH AMERICAN MAMMALS AND MOSQUITOES¹

III. FURTHER OBSERVATIONS ON *Haemagogus equinus* AS A VECTOR OF THE VIRUS

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In the first paper of this series the transmission of yellow fever virus by *Haemagogus equinus* was reported (1). Observations have been extended, and a new procedure has been developed for gauging the relative efficiency of different species of mosquitoes as vectors of the virus.

In addition to the innate quality of certain species of mosquitoes which makes them potential vectors of yellow fever virus, there are other factors which affect their chances of becoming infected with the virus and their ability to transfer the infection to a susceptible vertebrate host. It is recognized that the probability of a mosquito's becoming infected by feeding on an animal harboring yellow fever virus is related to the concentration of the virus in the blood of the animal at the time of the feeding, and that the multiplication of the virus in the mosquito, as well as the ability of the mosquito to transmit the infection by bite to a susceptible animal, are conditional on the length of the incubation period following the infectious meal and the temperature at which the mosquitoes are kept during this period of incubation (2, 3). It is not unlikely that other factors also play a rôle, such as the virus strain employed and the stage of the infection in the animal at the time the mosquitoes feed. For example, there is evidence indicating that the French neurotropic strain of yellow fever virus is more difficult to transmit through the bite of a mosquito than are the viscerotropic strains (4). Whether or not some viscerotropic strains may be more easily transmitted by mosquitoes than others has not been determined; but since viscerotropic strains of jungle origin manifest differences in their affinity for vertebrate hosts (5), it is conceivable that similar variations may exist in regard to their transmission by arthropod vectors. It has been noted, particularly when the titer of the circulating virus in the animal host is low, that mosquitoes may be more frequently infected when fed upon an animal during the early stage of the disease than later when the circulating virus is on the wane (6). This has been attributed to the development of antibodies that curtail transmission of the infection to the vector. It should also be recognized that in recently isolated strains which have not undergone passage in mice, the virulence of the virus for this animal may be relatively low, and it is therefore difficult to determine the concentration of the virus accurately by the usual mouse titration technique.

¹ The work on which these observations are based was done under the auspices of the Serviço de Estudos e Pesquisas sobre a Febre Amarela (Yellow Fever Research Service), which is maintained jointly by the Ministry of Education and Health of Brazil and the International Health Division of The Rockefeller Foundation.

It is evident, therefore, that in experiments designed to compare the relative efficacy of different species of mosquitoes as vectors, all of these factors must be taken into account and in so far as possible held constant. While certain conditions, such as the time of incubation following trial infection and temperature and humidity of the atmosphere in which the mosquitoes are retained during this period, can be predetermined and imposed, the important factors of the concentration of the circulating virus and the exact stage of the infection in the vertebrate host at the time the mosquitoes are exposed cannot be determined in advance. Consequently, it is virtually impossible to duplicate the conditions exactly in any two transmission experiments; and unless these conditions are the same the results may vary, irrespective of the vector employed.

In order to circumvent this contingency, in the second series of experiments here reported, a vector chosen as a standard for comparison was introduced and submitted to the same conditions as the vector under study. The efficacy of the latter was then determined by comparing its transmission ratio with that of the standard vector.

MATERIALS AND METHODS

Virus strain. The Olympio Christo (O.C.) strain was used. This strain was isolated from a non-fatal human infection contracted during an epidemic of jungle yellow fever (5). It had been passaged exclusively in rhesus monkeys and was in its seventh consecutive monkey passage.

Vertebrate hosts. Marmosets of the species *Callithrix aurita* E. Geoffroy or *Callithrix penicillata* E. Geoffroy were used as vertebrate hosts.

Insect vectors. Two species of mosquitoes, *Haemagogus equinus* Theobald and *Aedes aegypti* Linnaeus, were employed as insect vectors, the latter as a standard control for purposes of comparison.

Specimens of both species came from laboratory colonies. The *H. equinus* colony was initiated from eggs obtained from Osorno-Mesa (7) of Bogotá, Colombia, and the *A. aegypti* colony was established in 1942 from female mosquitoes captured at Nova Iguassu, State of Rio de Janeiro, Brazil.

Virus determinations. The presence and concentration of the virus in the blood of the vertebrate hosts at the time the mosquitoes obtained their infectious meal was determined by withdrawing blood either from the femoral vein or by heart puncture and inoculating, intracerebrally, decimal dilutions of the blood serum into groups of six white Swiss mice 21 to 28 days of age. The dilutions were made in 0.85 NaCl containing 10 per cent normal human serum.

EXPERIMENTAL

EXPERIMENT I. In a preceding communication (1) the alternate cyclic passage of yellow fever virus through *H. equinus* and *C. aurita* was reported. Three cycles were completed when the passages were discontinued for lack of an adequate supply of mosquitoes. After the *H. equinus* colony had become more prolific, another series of passages was initiated.

Technique employed in host-vector cycle. The technique has been described previously (1). In brief, it consisted of permitting the mosquitoes to feed upon an infected animal on successive days, usually between the second and fifth days following trial infection. At the same time blood was withdrawn from the animal and the amount of circulating virus determined as above indicated. After a suitable incubation period the mosquitoes (*H. equinus*) that had fed were allowed to bite a normal animal, which in turn was exposed to another batch of normal mosquitoes. Thus the cycles were continued. During the incubation period the mosquitoes were kept at a temperature of 28 to 30° C.

In this latter series of passages seven cycles were successfully completed before they were voluntarily discontinued.

The number of mosquitoes used in each passage varied from six to eighteen, the average being twelve. The incubation period between the infective meal and exposure to the next normal marmoset in the series was from 12 to 15 days. All of the exposed marmosets became infected. The maximum titer of the circulating virus, when determined, ranged from $10^{-6.6}$ to 10^{-8} .

EXPERIMENT II. While the foregoing experiment further established that *H. equinus* may serve as a vector of yellow fever virus in cyclic passages through marmosets, it did not reveal the percentage of mosquitoes infected, as one or all of the mosquitoes in each batch may have transmitted the virus. With the object of obtaining more exact information upon the efficacy of *H. equinus* as a vector the following experiments were performed.

Since it has been shown that baby mice are highly susceptible to extraneural inoculation of the virus and are readily infected by the bite of a mosquito containing virus in its salivary secretion, the use of this animal makes it feasible to test the mosquitoes individually (8).

Infected marmosets were exposed at the same time to normal *H. equinus* and *A. aegypti*. Immediately before the mosquitoes were allowed to feed, a small quantity of the blood was withdrawn from the animal and the virus content of the separated serum was determined. The mosquitoes which imbibed blood were segregated and stored in an atmosphere having a temperature of 28-30° C. and a humidity of 70 to 90 per cent. On the 14th, 18th, and 21st day thereafter they were permitted to feed individually upon baby mice 2 to 3 days of age. In these latter feedings to test the ability of the mosquitoes to transmit the infection, they were paired according to species. That is, for each surviving *H. equinus* an *A. aegypti* that had taken the infectious meal at the same time was likewise tested. The mice were kept under observation, and those which did not succumb within the expected period (7-20 days) were challenged for immunity on the 30th day by the intracerebral inoculation of 10,000 LD₅₀ French neurotropic virus. Table 1 reflects the results obtained.

The number of mosquitoes tested following 18 and 21 days' incubation is small, owing to the high mortality of *H. equinus*. It will be noted, however, that in each instance the ratio of transmission is higher with *A. aegypti* than with *H. equinus*. Following a 14-day incubation period, 37.1 per cent of the *A. aegypti* transmitted the infection to mice, as determined by death of the

animals or their subsequent immunity, as compared with 19.7 per cent of *H. equinus*. The difference is statistically significant ($P > .01$). Likewise there is a significant difference in the total ratio of transmissions between *A. aegypti* (43.9 per cent) and *H. equinus* (23.3 per cent) ($P > .01$) irrespective of length of incubation period.

TABLE 1

Comparative Infectivity of A. aegypti and H. equinus Fed Simultaneously on the Same Animals

MOSQUITO SPECIES	INTERVAL IN DAYS FOLLOWING INFECTIOUS MEAL						TOTAL TRANSMISSIONS REGARDLESS OF INCUBATION PERIOD	
	14		18		21			
	MR*	IR†	MR	IR	MR	IR	MR	IR
<i>A. aegypti</i>	43/132	49/132	14/21	16/21	7/11	7/11	64/164	72/164
<i>H. equinus</i>	21/132	26/132	8/20†	9/20†	3/11	3/11	32/163	38/163

* MR = Numerator represents the number of mice succumbing to infection. Denominator represents the number of mice fed on or probed by one mosquito.

† IR = Numerator represents the number of mice succumbing to infection plus those which resisted two intracerebral inoculations of 10,000 LD₅₀ of French neurotropic virus. Denominator as in MR.

‡ One of the mice died from an extraneous cause.

TABLE 2

Effect of Virus Concentration in Source Animal on Infectivity of A. aegypti and H. equinus

SERUM TITER AT TIME OF FEEDING	10 ^{2.9} - 10 ^{5.9}						10 ^{6.9} - 10 ^{7.9}						10 ^{8.9} OR OVER							
	14		18		14-18		14		21		14-21		14		18		21		14-21	
INCUBATION FOLLOWING FEEDING: DAYS																				
MORTALITY AND INFECTION RATIOS	MR*	IR*	MR	IR	MR	IR	MR	IR	MR	IR	MR	IR	MR	IR	MR	IR	MR	IR	MR	IR
Mosquito species																				
<i>A. aegypti</i>	1/44	1/44	0/3	0/3	1/47	1/47	10/37	14/37	4/5	4/5	14/42	18/42	31/47	33/47	9/11	9/11	3/3	3/3	43/61	45/61
<i>H. equinus</i>	0/44	0/44	1/3	1/3	1/47	1/47	12/37	14/37	2/5	2/5	14/42	16/42	8/47	11/47	7/10†	7/10†	1/3	1/3	16/60†	19/60†

* For explanation of MR and IR see footnote Table 1.

† One mouse died due to extraneous cause.

The titer of the circulating virus in the animals at the time the mosquitoes were fed varied from 10^{2.9} to over 10⁸. When the titer of circulating virus in the source animal was low at the time of feeding, few mosquitoes transmitted the infection. Thus, only one each of 47 *H. equinus* and 47 *A. aegypti* transmitted when the titer ranged between 10^{2.9} and 10^{5.9} (Table 2). A greater proportion, 16 of 42 *H. equinus* and 18 of 42 *A. aegypti*, transmitted when the virus titer was between 10⁶ and 10^{7.9}. Within these ranges of virus titer there

was practically no difference in the transmission rates of the two species. Contrary to expectation, the principal differences in the transmission rates occurred when the virus titer in the source animal was high, as 45 of 61 *A. aegypti* but only 19 of 60 *H. equinus* transmitted when the titer was 10^8 or over.

DISCUSSION

In previous experiments on the transmission of yellow fever virus by sylvan mosquitoes, the tests were made with primates, using as a rule a number of mosquitoes for each transmission, or if the mosquitoes were tested individually by the use of baby mice, no adequate control was included which would permit accurate comparison of the relative efficacy of different species as vectors of the virus. Permitting batches of mosquitoes to feed upon the same animal, when testing their ability to transmit the infection, obviously does not reveal the percentage of mosquitoes infected. Although testing the mosquitoes individually upon baby mice does permit determination of the percentage that transmit in a given observation, it does not warrant comparison with the results obtained in other experiments by reason of the uncontrollable variables peculiar to each transmission experiment. By including a standard vector and submitting it to the same experimental conditions as the species under investigation, and by establishing the ratio of transmission between the standard and the experimental vector, a more precise estimation of the efficacy of a vector should be obtainable than previous methods have allowed.

The known ability of *A. aegypti* to transmit the virus and the ease of maintaining a colony in the laboratory were the reasons for selecting it as the standard vector, but since different strains of this species may vary in their capacity to transmit the virus, it would be advisable to employ the same strain in all such experiments. It may be argued that the ratio of transmission observed in the laboratory between *A. aegypti* and sylvan species of mosquitoes would not necessarily apply in nature, as the environment of the laboratory is much more foreign to sylvan mosquitoes than to *A. aegypti*. Nonetheless, there is no apparent reason why such ratios should not serve as a basis for comparing one sylvan species with another.

In this instance 43.9 per cent of *A. aegypti* and 23.3 per cent of *H. equinus* transmitted the virus, and if a transmission index of one is assumed for *A. aegypti*, it follows that the index of *H. equinus* is 0.53.

Why the difference in the rate of transmission of *A. aegypti* and *H. equinus* was evidenced principally when the titer of the virus in the source animal was high is not clear. Indeed one would expect the reverse, that is, that a less efficient vector would transmit comparatively less frequently when the concentration of the virus in the source meal was low than when it was high.

In the studies of Anderson and Osorno-Mesa (3) on *H. splendens* and for the most part in those of Bates and Roca-Garcia (2) on *H. spegazzinii falco*, the body of the mosquito was triturated and the resulting suspension inoculated intracerebrally into adult or baby mice. By this procedure a higher percentage of mosquitoes can be shown to retain the virus than are able to transmit the

infection by bite, especially if the tests are made following a relatively short incubation period. The results are therefore not comparable with the experiments here reported. From the epidemiological aspect, transmission of the virus by bite is the feature of essential importance.

SUMMARY

A strain of jungle yellow fever virus was maintained by alternate passage through marmosets and *Haemagogus equinus* for seven cycles, after which passages were voluntarily discontinued.

A comparison was made between the efficacy of *H. equinus* and *A. aegypti* as vectors of the virus, by submitting the two species to the same experimental conditions and by testing the mosquitoes individually through allowing them to feed upon baby mice at varying periods following the infective meal.

It was found that under the imposed experimental conditions 72 of 164 *A. aegypti* and 38 of 163 *H. equinus* transmitted the virus, thus giving a transmission of 43.9 and 23.3 per cent respectively, or an index of 0.53 for *H. equinus* as compared with an assumed index of 1 for *A. aegypti*.

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PSYCHOTIC REACTIONS TO THE INGESTION OF LARGE DOSES OF QUINACRINE IN NORMAL SUBJECTS

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Psychic disturbances following the treatment of malaria with quinacrine have been reported by a number of authors.¹⁻⁴ In such instances it has usually been impossible to exclude as the cause of the mental disturbance the malarial fever itself or some hypothetical toxic substance resulting from the rapid destruction of a large number of parasites by quinacrine. In the present report malaria could be positively excluded as a factor in a number of individuals who developed severe toxic mental reactions following the ingestion of large doses of quinacrine.

PROCEDURE

Thirty-one medical, dental and medical administrative corps officers of a United States Army General Hospital volunteered to take quinacrine at first in suppressive and then in therapeutic dosage. None of these officers gave a history of having had malaria, and all of them had been stationed both before and throughout the observation period in a non-endemic area. The dosage schedule was as follows: Quinacrine hydrochloride 0.1 gram daily for 1-2 weeks, 0.1 gram twice daily for one week, and concluded by 0.4 gram three times the next day, 0.3 gram three times the following day, and 0.1 gram thrice daily for the remaining four days.

Following the ingestion of the large dose of quinacrine, it soon became apparent that certain cerebral symptoms were developing in a large percentage of the officer personnel. The thirty-one individuals in this experimental group were carefully questioned as to their symptomatology and were compared with five control subjects in whom the quinacrine had been replaced by placebos. The control subjects never reported more than mild central nervous system symptoms such as restless sleep, tension, etc. This observation, coupled with the fact that the experimental subjects exhibited no significant symptoms before and some days after the ingestion of toxic doses of quinacrine, furnished evidence that all the reactions recorded herein were attributable to the effect of the drug. The fact that the mental reactions occurred quite unexpectedly adds further support to the belief that the symptoms to be described can be attributed to the pharmacological effect of the drug alone.

OBSERVATIONS

Mental disturbances occurred in one form or another in 24 of the 31 officers taking the medication. Increased dreaming and insomnia were common. Severe psychic disturbances occurred in twelve individuals, three of whom developed frank psychoses with delusions, hallucinations, and profound changes in mood.

Sleep was affected in 58 per cent, with an estimated maximal reduction in

sleep obtained to one-half of normal for the individual. In eight instances this was so severe that the subject voluntarily sought relief by the self-administration of sedatives with varying effect on the insomnia. Twenty-two, or 70 per cent, of the officers complained of dreams which, they said, were ordinarily so rare as to make their occurrence under quinacrine administration more than coincidental. These were rarely terrifying dreams, more often anxious and disturbing, and occasionally pleasant. Frankly sexual content was not common.

Minor psychic complaints also occurred in a number of the subjects, the most notable being a feeling of increased tension (11 cases), restlessness (10 cases), tremor (9 cases), overtalkativeness (3 cases), overactivity (4 cases), headache (3 cases), chilly sensations (2 cases), increased perspiration (1 case). These were commonly noticed first after the larger doses, but in three instances they were described during the latter half of the week in which 0.1 gram twice daily was taken.

Mood changes occurred in 14 subjects. There were 9 who experienced mild feelings of depression while five noted elation. One of those who became depressed contemplated suicide but immediately recognized the futility of such an act. Most of the depressions were relatively mild and accompanied by considerable insight. The sense of elation and well-being was often spectacular, two of the subjects singing spontaneously for an hour or so on several occasions.

Disturbances in the content of thought were varied and most striking. Several officers commented on acquiring rapidity and ease of thinking, heightened appreciation of music, ability to write unusually lucid and expressive letters, lack of fatigability, impulsiveness, overproductive imagination resulting in grandiose planning, increased libido, and a general sense of euphoria comparable to the effects of benzedrine or to moderate doses of alcohol. One person, for example, spent four hours in writing long letters home outlining detailed plans for his post-war life; another made proposals to buy large plots of land adjacent to his home. In general, these responses tended to exaggerate, enlarge, or project the basic emotional and thought content which existed prior to taking quinacrine.

Frank psychotic trends developed in three individuals. One had the idea he was commanding officer of a large general hospital, and although he recognized vaguely that he was deluded, he could not entirely shake the idea and kept making plans in accordance with his imagined responsibilities. Another, who was ordinarily neat and extremely careful and courteous, became confused, left his quarters in extreme disorder, made mistakes in paying bills and accused various tradesmen of cheating him. He thought the hospital was to move the next day, and packed all his equipment in a burst of overactivity. The third, ordinarily quiet and cooperative, became aggressive, ordered his friends about and refused to accept criticism or advice. He stayed in bed all day, demanding that others run errands for him while he sang hymns and expressed grandiose ideas about what he would do on his return home. Later he became frankly disoriented and excited, had to be hospitalized and later transferred to a closed

ward where he developed numerous delusions such as that he was being tortured in a Nazi prison camp. In all three cases partial insight was always present; there was little or no amnesia, and discontinuing the quinacrine resulted in rapid improvement.

A careful history revealed that in 9 cases mild mental complaints began after about four or five days on the 0.2 gram daily doses. Reduced sleep or disturbed dreams were mentioned by 8 individuals even before the larger amounts of quinacrine were taken. Tension and overactivity were early symptoms in 1 case, and 2 noticed some depression. In general these disturbances were very mild before the higher dosage was reached. The early development of these symptoms did not necessarily occur in those who later became most severely affected. Of the 9 quoted above, only 4 subsequently were classified as having severe mental reactions, whereas 8 others developed marked symptoms only after ingestion of the larger doses. In mild cases the maximum effect occurred from 8-48 hours after taking the first of the 0.4 gram doses of quinacrine, and subsided within 1-3 days after stopping the medication. However, in the 2 most severe cases symptoms reached their peak in 3 and 6-10 days respectively, after the largest dose had been taken, and cessation of symptoms was correspondingly prolonged by another 4-14 days. In general, the most reliable warning sign was insomnia or increased dreaming, which always preceded the development of the more severe reactions. Gastro-intestinal complaints usually appeared before other symptoms and were prominent in most of those who developed the more profound mental disturbances. However, they did not prove to be a reliable early guide to toxic mental effects, since in many who subsequently exhibited only a minimal psychic response, gastro-intestinal symptoms were most distressing.

Although many did not complete the planned final doses of 0.1 gram thrice daily for 4 days, only 5 officers failed to complete the prescribed dosage of 2.1 grams in 2 days, the exceptions being in those individuals who experienced the most disturbing mental reactions to ingestion of 1.2 grams on the first day, and voluntarily discontinued the medication thereafter. One subject took more than the prescribed amount; namely, 0.5 gram instead of 0.3 gram on the third day of the intensive dosage, and had taken 0.1 gram for two weeks instead of one at the beginning of the experiment. It was this person who exhibited the most severe mental aberrations. In view of the many officers who were able to complete their course with milder or no symptoms at all, it would seem that mental reactions tended to occur in the more sensitive individuals regardless of total dosage. In this connection it is interesting that two of the more severely affected gave a preceding history of a mild depressional episode.

DISCUSSION

The high incidence of mental reactions to quinacrine was most unexpected, since the drug had been administered in only slightly lesser dosage to hundreds of malarial patients in the hospital and only one probably psychotic reaction had been recorded. It must be conceded, however, that minor toxic symptoms

of restlessness, insomnia, and increased dreaming might frequently have passed unnoticed as a manifestation of malaria.

It would appear that at least three factors might play a part in explaining the foregoing observations: dosage, tolerance, and individual susceptibility. Routine therapeutic dosage for malaria in the hospital for a considerable period corresponded to that given the volunteers, but at the start of the intensive therapeutic program the patients may be assumed to have had relatively low blood levels since they had relapsed, while the experimental subjects who had just completed a week of quinacrine 0.2 gram daily should have had a substantially higher blood concentration of the drug. Consequently, following a standard therapeutic dose the volunteer group could be expected to have attained significantly higher blood levels and to have shown a greater incidence of toxic reactions. Another difference between the two groups consisted in the fact that the patients had received suppressive quinacrine over varying periods of time prior to taking the large doses, while the volunteers, having taken no quinacrine previously, had had no opportunity to acquire a tolerance to the drug. Finally, individual variation undoubtedly played a role, since the most toxic reactions occurred in two persons with a previous history of depressional episodes. That these effects were more than a manifestation of personal idiosyncrasy to the drug seems likely from the fact that 78 per cent of the volunteers reported some form of mental disturbance, that all gradations from mild to most severe reactions were encountered, and that the worst and most prolonged effect was experienced by the officer who took the largest doses.

It would seem probable from these observations that in individuals who have not previously received quinacrine, the rapid development of high blood levels such as were probably attained in this experiment after the administration of 0.1 gram daily for one week, 0.2 gram daily for one week, and 1.2 and 0.9 gram on subsequent days approaches the threshold of cerebral toxicity for this drug. The use of massive daily doses of quinacrine therapeutically in patients who have been on high suppressive dosage may result in unpleasant reactions. Insomnia, restlessness, increased dreaming and mild depression or elation are the warning signs of an impending psychotic reaction. Finally, this report clearly establishes that the drug alone, without concurrent malaria, may be responsible for the signs of mental toxicity which have been frequently reported in the literature.

CONCLUSIONS

a. Twenty-four of thirty-one officers, taking for the first time doses of quinacrine moderately in excess of those used in routine anti-malarial therapy, developed toxic central nervous system symptoms varying from mild insomnia and depression to severe psychotic reactions in three instances.

b. Since malaria was definitely excluded in every instance, and the reaction subsided promptly upon discontinuing the drug, quinacrine alone could be definitely implicated as the cause of the psychic reactions encountered.

c. It is probable that in the dosage schedule here employed, in subjects un-

as related to the drug, the threshold for cerebral toxicity for quinacrine in the human being was approached, and that its earliest manifestations are insomnia, with or without increased dreaming and mild depression or elation.

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THE LYMPH NODE IN TROPICAL DISEASES

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The lymph node is such a prominent feature, and is of such diagnostic potentiality in so many tropical diseases that a review and correlation of its reactions may be of some value. This review does not include the minutiae of the pathologic pictures possible in the lymph node in the various tropical diseases, nor is it concerned with the discussion of the diseases themselves. The detailed pathology of tropical diseases, including those in which lymph nodes are prominently involved such as filariasis, have been covered by recent publications. The purpose of this paper is simply to emphasize the significant features in the lymph nodes from those diseases in which that structure is conspicuously involved.

It is possible to group these diseases in several ways. One would include those in which the defense mechanism is phagocytosis by the reticuloendothelial system: Oroya fever, histoplasmosis, visceral leishmaniasis and malaria. Another group could include those in which the dominant clinical and pathologic feature is centered in the lymph nodes. These would be lymphopathia venereum, plague, typhoid fever and filariasis (*Wuchereria*). A third possibility comprises those diseases in which the lymph node is incidentally but significantly involved. These diseases would be histoplasmosis, visceral leishmaniasis, typhoid fever, rickettsial diseases, brucellosis and leprosy. A sub-classification, based on the pathogenesis, would bring together such strange bed-fellows as filariasis and rickettsial diseases. In both of these, it is not so much the lymphatic tissue *per se* that is of significance, but lesions of the node vessels. An additional sub-group would include those diseases in which the lymphatic tissue *per se* is specifically, but not exclusively involved. These diseases are lymphopathia venereum, typhoid fever, and brucellosis. Finally, there can be a histologic differentiation on the basis of the type of endothelial response. First, those in which the phagocytosis is predominantly by the vascular endothelium. These would be the rickettsial diseases and Oroya fever. Secondly, those in which there is diffuse proliferation of the reticuloendothelial phagocytes: histoplasmosis, visceral leishmaniasis, leprosy, and, to a lesser extent, malaria. Thirdly, those in which granuloma is a prominent feature. These are lymphopathia venereum, typhoid fever, filariasis and brucellosis. It will be noted that none of these groupings depends upon type of etiology whether bacterial, protozoal, viral, rickettsial, or helminthal, and that one disease may belong in several groups. It is only, therefore, in those lymph nodes in which the specific infecting agent is recognizable that an unequivocal diagnosis can be made from histopathology alone, although in plague and typhoid fever the histology is sufficiently characteristic to warrant strongly presumptive diagnosis, even ignoring the abundant bacterial content in the former, itself a distinctive characteristic. The granuloma of lymphopathia venereum cannot be differ-

entiated morphologically from that of tularemia for example, and at times we see a similar stellate granulomatous abscess in filariasis without an obvious worm. Superficially, the diffuse macrophagic reaction in histoplasmosis, leishmaniasis and leprosy are very similar, and may even simulate the picture in the lipoid histiocytoses, such as Gaucher's disease. The reticuloendothelial reaction in brucellosis is at times bizarre, suggesting the picture in Hodgkin's disease, a suggestion that has been taken so seriously by some observers as to lead them to propose *Brucella* infection as a cause of Hodgkin's disease.

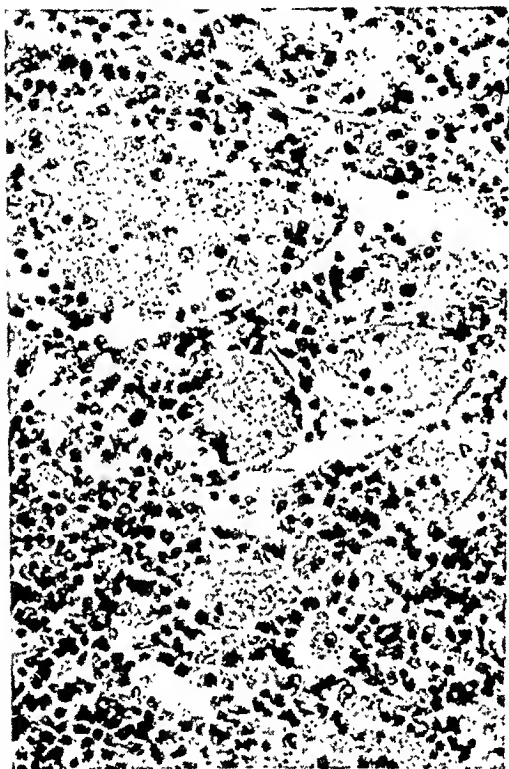


FIG. 1. Visceral leishmaniasis

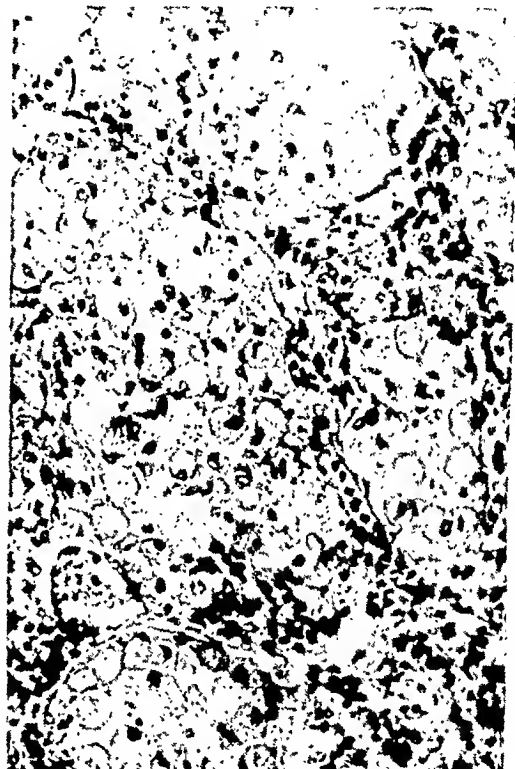


FIG. 2. Gaucher's Disease

FIGS. 1 AND 2. DIFFUSE RETICULOCYTIC REACTIONS IN LYMPH NODES

It will be noted that a common factor in all of the diseases included in the study, even plague, although to a lesser degree than the others, is the macrophage of whatever histogenesis. While predominantly from reticuloendothelium, in some instances, such as Oroya fever, rickettsial diseases and malaria, the basophilic macrophage is of lymph-cell origin. Exudative reaction is not a prominent feature in any of the group except plague and, to a lesser extent, lymphopathia venereum and brucellosis. Of all the conditions, the least distinguishable, on the basis of lymph node changes alone, are the rickettsial diseases. The pathologic changes limited to vascular lesions, so far as specific features are concerned, can be simulated by the other specific vascular diseases, such as disseminated lupus erythematosus and periarteritis nodosa. This is particularly true of endemic

typhus and Rocky Mountain spotted fever, in which fibrinoid necrosis is a prominent feature, but it is absent in scrub typhus.

Following is a summary of the essential features of the individual diseases included in this review:

Lymphopathia venereum (climatic bubo, tropical bubo). This is one clinical lymph node disease that has been removed from the limbo of etiologic uncertainty by the recognition that it is a specific viral disease. Before this discovery in 1930, the term undoubtedly was applied to a variety of the adenopathies common in the tropics. Castellani was a bit ahead of his day, for a

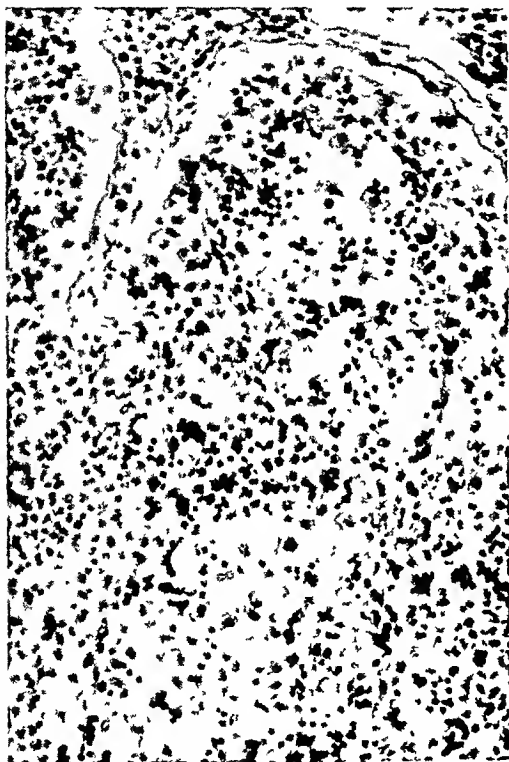


FIG. 3. Leprosy

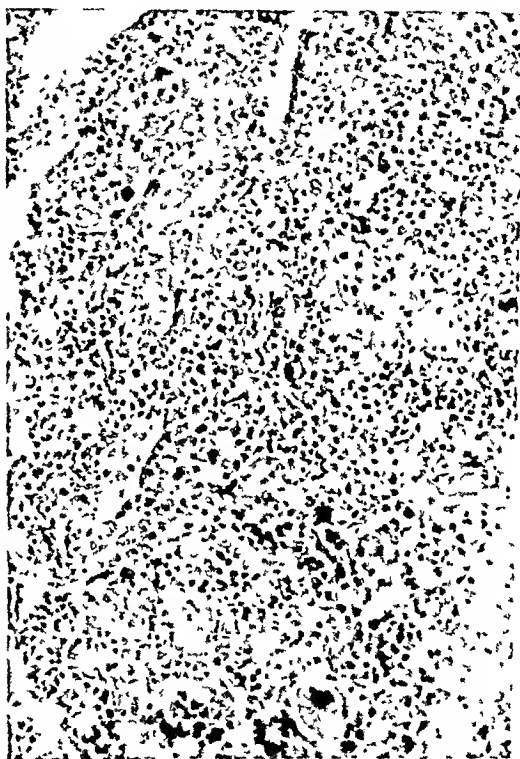


FIG. 4. Histoplasmosis

FIGS. 3 AND 4. DIFFUSE RETICULOCYTIC REACTIONS IN LYMPH NODES

number of years before the isolation of the virus he had rejected the commonly held idea that it was an attenuated form of plague, although he considered it a specific disease. He did not, however, consider it of venereal origin. The venereal bubo is a complication of soft chancre. It is the single tropical disease practically limited to the lymphatic tissue. This holds true in the common pelvic and rectal complication in women. The histology, while characteristic, is not sufficiently so to establish the diagnosis. The stellate abscess, a characteristic feature of in the picture, cannot be differentiated from the granuloma of several other infections, particularly tularemia. It is closely simulated at times in filarial infection, and the difficulty in differentiation is increased when

the worm is absent, and when the lymphopathia abscess contains a preponderance of eosinophilic leukocytes, as it sometimes does. The granuloma is readily distinguishable from that of tuberculosis and syphilis by the type of necrosis, in addition to the demonstration of the causative organisms in these diseases. The necrotic areas in lymphopathia contain polymorphonuclear leukocytes and chromatin debris not found in the other two tubercles. The zone of palisaded histiocytes in which Langhans giant cells are common, the surrounding zone of



FIG. 5

FIG. 5. STELLATE GRANULOMA OF LYMPHO PATHIA VEREREUM



FIG. 6

FIG. 6. STELLATE GRANULOMA OF FILARIASIS (*W. bancrofti*)

lymphocytes and plasma cells, and the secondary fibrosis, are not specific. In lymphopathia there may occasionally be seen small acidophilic or basophilic intracytoplasmic inclusions, the gamma-Favre bodies. It is questionable that they can be considered pathognomonic.

Granuloma inguinale is not a disease of lymph nodes, and is not included in this review, in spite of the confusion in names and the fact that it is also venereally transmitted, and that it has clinical similarities to lymphopathia.

The *Rickettsial diseases* specifically do not involve the lymph nodes, but they do cause the same vascular damage in them as is seen in other tissues. In scrub typhus there is usually a generalized lymph node enlargement, and the nodes draining the area of the primary lesion may be greatly enlarged and show gross

areas of necrosis. The sinuses are packed with inflammatory cells, the most prominent being the large basophilic macrophage which is the common denominator of the lesions produced by all of the rickettsiae. The exudate usually extends beyond the capsule into the surrounding tissue. Necrosis, at times extensive, particularly near the medulla of the node, is the result of the vascular damage. In this connection it is well to call attention to the differentiating point in the arteritis of scrub typhus and that of epidemic typhus and of Rocky Mountain spotted fever. The fibrinoid necrosis of the arterial and periarterial collagen is common in the last two diseases and, incidentally, also in such specific arterial diseases as periarteritis nodosa, disseminated lupus erythematosus and allergic response to the sulfonamides. It is not ordinarily seen in scrub typhus.

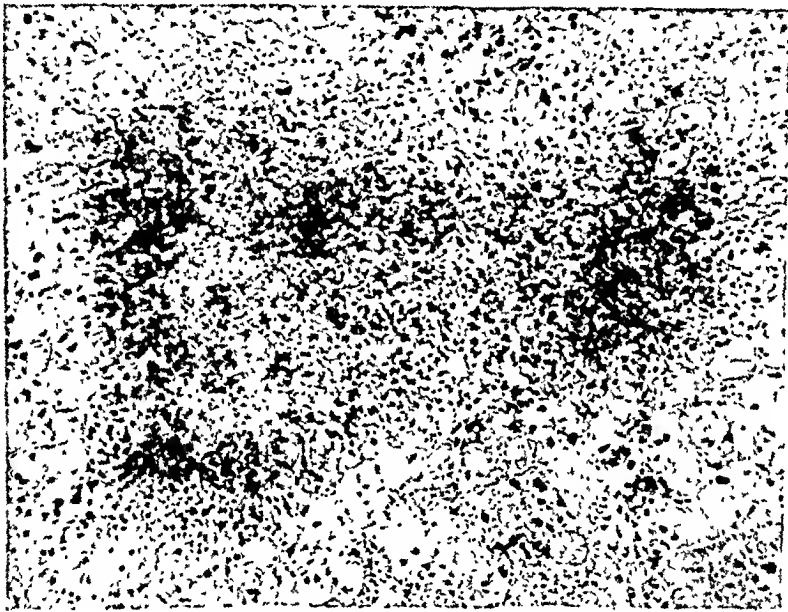


FIG. 7. GRANULOMA OF HISTOPLASMOSIS

The large basophilic macrophage mentioned is non-specific to scrub typhus or even to the rickettsial infections generally, and is encountered in a variety of reactions.

In *Oroya fever*, the acute febrile phase of bartonellosis, the lymph nodes are specifically concerned, in addition to the red blood cells, because of the involvement of the reticuloendothelial system as well as the vascular endothelium. The vascular endothelium becomes enormously enlarged from the phagocytosis of masses of *Bartonella*. While these masses are obvious with ordinary stains, it is necessary to use special bacterial stains to demonstrate the details. The involvement of the endothelium sometimes leads to occlusion of the vessels with resulting infarction. This is more commonly seen in the spleen than in the lymph node, however. A less specific histopathologic feature is the "sinus catarrh". The sinuses are dilated, the endothelium swollen and the lumina

filled with lymphocytes and very large macrophages. In addition to the organisms, hemosiderin may be demonstrated in the phagocytes, evidence of the red-cell destruction.

It is not necessary to discuss the lymphadenopathy of the tropical treponematoses, except to say that it is evident in all four diseases, syphilis, yaws, bejel and pinta, most characteristically in yaws and syphilis. The histopathol-

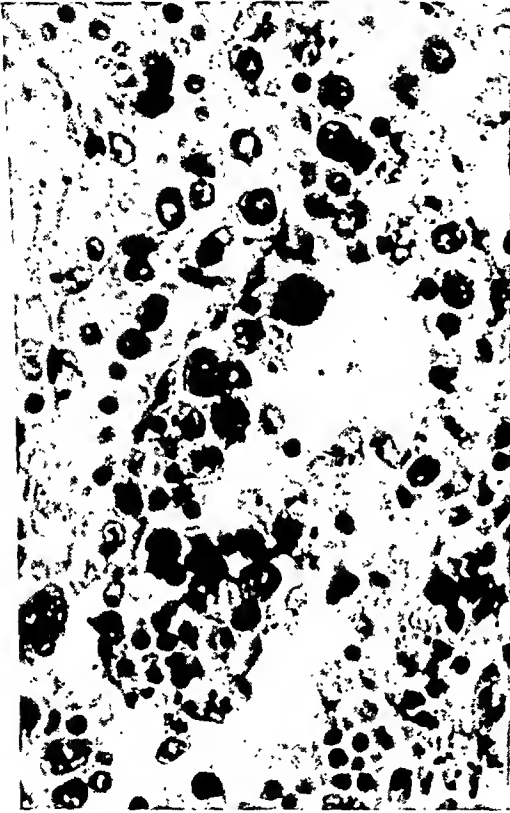


FIG. 8



FIG. 9

FIG. 8. BASOPHILIC MACROPHAGE IN SCRUB TYPUS

FIG. 9. VASCULAR ENDOTHELIAL REACTIONS IN OROYA FEVER

ogy is practically the same in the four diseases, and while the respective *Treponema* are demonstrable in the lymph nodes in all four, it is not much help in differentiation, because the three species are indistinguishable morphologically.

Brucellosis. The reaction to *Brucella* by the lymphatic tissue is of great interest to the pathologist, and has led to considerable friendly controversy between those who have interpreted the bizarre reticuloendothelial reaction as Hodgkin's disease and those who have recognized it as a non-specific and non-neoplastic reaction. This reaction is seen in a variety of infections and stimulating influences, especially irradiation. It is one of the most striking features in the lymphatic tissues of the Japanese A-bomb victims. This reaction in

Brucella infections is seen more particularly in the mesenteric lymph nodes and spleen, and most commonly in the *melitensis* infection, and includes bizarre multinucleated cells that are indistinguishable at times from the Reed-Sternberg cell of Hodgkin's disease.

Typhoid fever is a lymphadenotropic infection with a reaction that is usually sufficiently characteristic to warrant identification in the absence of other criteria. Typhoid fever is one of the acute infections in which the reaction is proliferative rather than exudative. The typhoid cell is a large mononuclear cell which tends to collect in foci and to displace the normal lymphatic tissue. These cells are actively phagocytic, engulfing bacteria, cells and cell detritus.

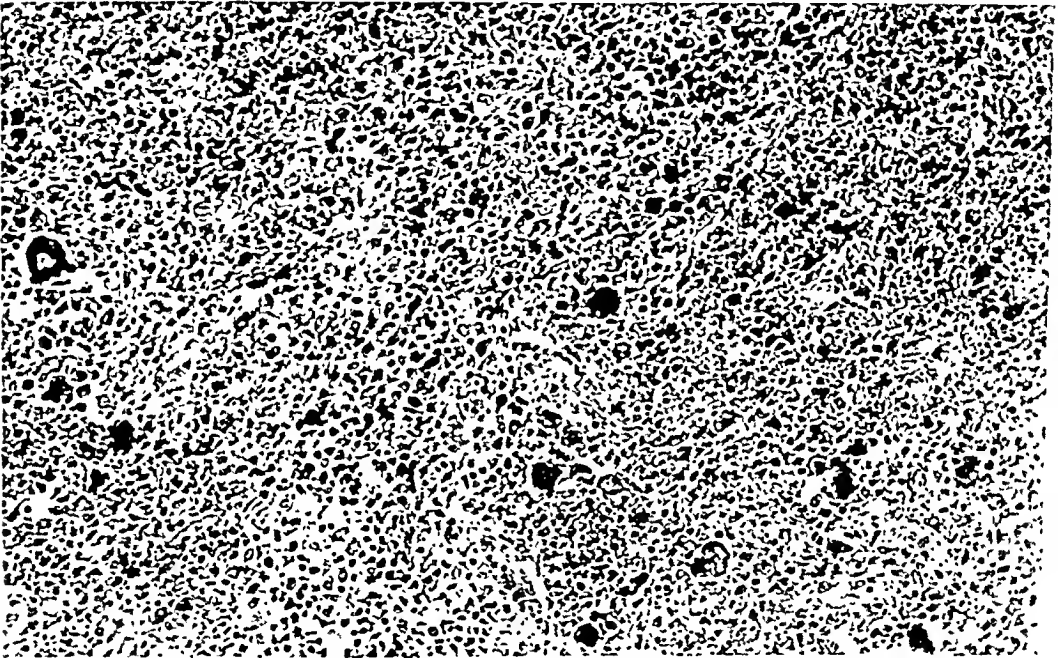


FIG. 10. HODGKIN'S DISEASE—LIKE REACTION ON BRUCELLOSIS

The typhoid granuloma differs from others in the absence of giant cells, polymorphonuclear exudate, and by the karyolytic type of necrosis. Of course, it is the mesenteric lymph nodes that are predominantly involved. Bacteria may also be demonstrated in the plasma cells.

Plague. While it is possible to divide plague clinically into the three forms, bubonic, pneumonic and septicemic, the bubonic form not only predominates in incidence, but significant bubos are usually present in the other two forms. The septicemic and pneumonic forms may, however, be so rapidly fatal that clinical bubo does not have an opportunity to develop. In all cases, however, there is localization of the *Pasteurella* in the lymph nodes. On the other hand, in the fatal cases of all forms there is practically always a terminal bacteremia. The primary localization of the organism is in the lymph node, except perhaps, in the pneumonic form resulting from droplet inhalation. The bubo, frequently

inguinal, becomes apparent on the second to fifth day of the disease. It is characterized clinically by great size, great pain and tenderness. The histology is sufficiently characteristic to warrant a diagnosis. The toxin of *P. pestis* causes necrosis of the vessel walls, and the reaction is characterized by extensive necrosis and hemorrhage with comparatively little exudate and great numbers of bacteria. Characteristically, the reaction, even in the early stages, extends beyond the lymph nodes, and results in a large edematous, hemorrhagic, necrotic mass of nodes and surrounding tissues.

Leprosy. It has been said by experienced leprologists that involvement of one or more of the peripheral lymph nodes is the most constant feature of leprosy, and that when it is not possible to establish a diagnosis bacteriologically from the usual sources of skin and nasal mucosa, it may be done by aspiration or biopsy of the lymph node, most commonly inguinal. Histologically, the picture again is sufficiently specific to be suggestive through the distribution of the characteristic lepra cell, and confirmatory through the presence of large numbers of bacteria. In the early stage the pigmented foam cells are scattered near the cortex, but contain bacilli. In the later stage, there is great diffusion through the node with characteristic fat-like, clear spaces that are the result of the vacuolization of the lepra cell. Involvement of the lymph node occurs in both lepromatous and neural types.

Fungus Diseases. We are including histoplasmosis from the group of the fungi, as being the only one of significance for our purpose, the only fungus that has a predilection for the reticuloendothelial system. Since the reaction is predominantly reticuloendothelial, with little inflammatory reaction, and with necrosis limited to the larger lesions, there may be confusion in differentiating histologically this infection from other endotheliotropic infections, especially early leishmaniasis and early leprosy. The necrotizing granulomas are seen more commonly in the intestine, adrenal and spleen. The differentiation between the *H. capsulatum* and *Leishmania* offers little difficulty to the experienced observer, although it is of interest to note that there are one or two cases of *Leishmania* reported in the literature as endemic in this country, that were later proved to be misdiagnosed histoplasmosis infections.

Leishmaniasis. *L. donovani* is another reticuloendotheliotropic parasite with a special affinity for the reticuloendothelium of the spleen. As a matter of fact, ordinarily the lymph node is not particularly involved except in heavy infections, and the reticuloendothelial cells are more scattered, and necrosis is rarely seen. These criteria might be accepted as differentiating points from histoplasmosis when only the lymph node is examined, and then there might be uncertainty as to the morphology of the organism. Incidentally, reaction in the spleen may suggest, on superficial examination, one of the lipoid histiocytoses.

Malaria can be dismissed with the notation, that in spite of the reticuloendothelial content of the lymph nodes, localization there of parasites is not particularly heavy, although pigment may be present. This relative sparsity of parasites in the lymph node can probably be accounted for by the difference

in blood supply in the lymph nodes, as compared with that in the spleen and the liver, where a larger quantity of blood in a sluggish flow is brought into contact with the phagocytes.

Filaria.—It is not necessary to go into the details of the pathology of the *Wuchereria* infections. These have been well-covered in the recent literature. The adult worms are found most commonly in the lymphatic vessels, particularly those draining the lower extremities with the additional clinical point that *W. bancrofti* may localize about the mammary gland or in the axilla. So long as the worms are viable and freely movable in the vessels, there is little reaction. When they die or become impacted in vessels, or when the larvae invade surrounding tissues, there is thrombosis and inflammatory reaction, both suppurative and granulomatous. There is little difficulty in identifying the cause of the reaction when the parasites are included in the lesions; but the lesions do not always contain worms or their fragments, and may then be difficult to interpret. These extravascular lesions may be the effect of toxins or of excretory products of the living worm. An interesting feature of the histology is that eosinophilia may be present in lymph nodes in which worms may not necessarily be identified. Concentration of eosinophils may reach the point where they form eosinophilic abscesses. The more scattered type of distribution of the eosinophilic cells in conjunction with reticuloendothelial reactions may be such as to suggest the diagnosis of Hodgkin's disease.

THE EFFECTIVENESS OF METACHLORIDINE IN SUPPRESSING NATURAL INFECTIONS WITH *PLASMODIUM MALARIAE* AND *P. FALCIPARUM* IN BRITISH GUIANA

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Metachloridine (2-Metanilamido-5-chloropyrimidine) was found to be the most active of an extensive series of metanilamide derivatives both in preventing and suppressing infections with *Plasmodium gallinaeum* in chickens (Brackett and Waletzky, 1946). This compound has also been shown to be highly active as a suppressant of blood-induced infections with *P. cathemerium* in canaries (Hughes and Brackett, 1946) and *P. cathemerium* and *P. lophurae* in ducks (Marshall, 1947). It also prevented sporozoite-induced infections with *P. cathemerium* in the canary (Hughes and Brackett, 1946; Gingrich, Schock and Taylor, 1946) and *P. lophurae* in the turkey (Porter, 1947).

Metachloridine received trial by Pullman and his colleagues (1947) and others in experimental vivax infections in human volunteers. Pullman found that a total of from 60 to 500 mg. administered over a four-day period, starting four days after onset of symptoms of blood-induced infections of two strains of *P. vivax*, caused a complete although temporary, disappearance of parasites from the bloodstream. Higher doses caused the permanent disappearance of these parasites, while doses of 20 to 60 mg. only caused a reduction in parasite numbers. These same investigators presented limited data suggesting that 0.25 gm., but not 0.125 gm., of metachloridine once weekly may be completely effective in suppressing sporozoite-induced infections with a South Pacific strain of *P. vivax*.

The trials of Pullman, of course, had been preceded by pharmacological and toxicological studies in animals (Robinson and Mayer, 1947) and man, and these indicated that man could tolerate doses of three grams of metachloridine a day for as long as 20 days without symptoms. Five grams a day produced hematuria in one patient. Thus it was believed that it would be safe to use the smaller doses that would probably be necessary to make the drug of practical interest in relation to other newer antimalarials such as paludrine and chlorquine. These are effective suppressants at least of *P. falciparum* and *P. vivax*, in doses of less than one gram given once weekly (Fairley, 1946) and (Goldsmith, K., 1946).

With the above data at hand it seemed justifiable to submit metachloridine to trial in the field in an attempt to determine its effectiveness in suppressing naturally acquired malaria. The sites chosen for these trials were several areas in British Guiana where malaria is endemic and generally of high incidence. This paper reports some of the results of these trials, which showed that infections with *P. malariae* can be completely suppressed with metachloridine, while the parasitemia at least, of *P. falciparum*, may be only incompletely suppressed with doses of 1 to 2 gm. of metachloridine weekly. Data regarding *P. vivax* were too

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limited to permit a definite conclusion but they did not conflict with the data of Pullman, *et al.* (loc. cit.)

METHODS AND MATERIAL

a. *Experimental Groups.* The most useful results were obtained in trials with school children in four schools in the neighborhood of New Amsterdam, British Guiana. Other groups were used but were not productive, due to the low incidence of malaria in the controls. Each school was located on a different sugar estate and the students were chiefly children of the laborers. The schools are referred to by the name of the estate (table 1). About 90 per cent or more of the children at Blairmont School were East Indians, 90 per cent or more at Providence and Edinburgh were Negroes and the two races were about equal in number at Friends school. A small number of Chinese, native Indians, and whites were found in these populations. These schools are located in a flat, low coastal area where a great deal of water, suitable for the breeding of *Anopheles darlingi*, the principal vector in the region, is found, particularly during the rainy seasons. There was no protection from mosquitoes for most of the population, the houses being open and unscreened. The incidence of positive blood smears may be as high as 75-80 per cent at certain times of the year (Giglioli).

Permission to conduct these trials was obtained from the Director of Medical Services and the Director of Education for the Colony, and confirmed by His Excellency, The Governor of British Guiana. Cooperation of the local teachers and estate managers was easily obtained. Dr. George Giglioli, Medical Director of the Sugar Growers Cooperative, and Honorary Government Malariologist, was extremely helpful in making records available to us, and in his recommendations.

Treatment. A total of 250 children was selected for treatment, and an equal number for controls. The children were selected at random, and the ages were equally distributed in the treated and control groups. Details are given in table 1. Treatment was conducted from February 4, 1946, to April 17, 1946, and from May 6 to July 26, 1946, the interruption being due to the Easter holiday. Treatment to May 30 was with a dose of about one gram each week, adult basis, divided into 2, 3 or 5 parts. That is, a child weighing about half the mean weight of an adult received 0.5 gm. per week, in some cases having this divided into two equal doses received on Tuesdays and Thursdays, while in other cases the drug was divided into 3 or 5 doses (table 1). On May 30, the dosage was doubled (table 1). The drug was administered by the head teacher in each school who had been carefully instructed.

Blood examinations. All blood smears, both thick and thin for each individual each time, were made by our own local technicians who had been carefully trained in this technique, as well as in microscopic diagnosis by one of us (M. K.). The slides were examined by these same men, but all positive smears were checked by us for identity of species and a regular examination of a sample of the negatives was made by us as a check on the care with which the slides were being examined. Thick smears were examined for 5 minutes before they were classified as negative.

THE EFFECTIVENESS OF METACHLORIDINE

TABLE I

OF METACHLORIDINE

TABLE I

SCHOOL		INCIDENT OF MALARIA					No children		Frequency of report	Dose of Metachloridine	Total No. of children treated	Total No. of children not treated				
		in Survey a Year Prior	at Onset of Epid. 2/1/16	9/15/16 50 Days after Last Treat- ment		per cent	in Treated Group	in Control Group								
		per cent	per cent	per cent	per cent	per cent	per cent	per cent								
Blairmont		42	15				100	100		5 lb. 5, 10, 15	100	100				
Providence		50	8	48			50	50	3 times per week Mon through Fri	ages 6-9, 50 mg ages 10-14, 100 mg	50	50				
Edinburgh		72	13				50	50	twice weekly Tues and Thurs	ages 5-9, 50 mg ages 10-14, 100 mg ages 15-19, 200 mg	50	50				
Friends		78	0	38			50	50	3 times per week	ages 5-9, 25 mg ages 10-14, 50 mg ages 15-19, 100 mg	50	50				
									3 times per week Mon, Wed, Fri.	ages 5-9, 50 mg ages 10-14, 100 mg ages 15-19, 200 mg	50	50				

* Approximate corresponding weekly dose for adult of 145 lbs. for period 5/30-7/28.
 † During the period 5/30-7/28.

* Approximate corresponding weekly dose for adult of 145 lbs. for period 5/20-7/20.
 † During the period 5/20-7/20.

Species identification was checked on the thin smear. The stages of development seen were recorded, and a rough attempt to quantitate the density of parasites was made. Only routine smears could be made since the available staff was not adequate for a daily check of absentees, and those complaining of suggestive symptoms. This may give a distorted picture of the incidence of malaria, since if complete suppression of clinical symptoms but only incomplete suppression of parasitemia occurred, the incidence would appear higher due to the presence at school of infected individuals, while failure to suppress clinical malaria would tend to result in an apparently lower incidence due to absence from school of infected individuals. Blood smears were made just before treatment was started, immediately after the Easter vacation, 50 days after treatment was terminated and at about 3 week intervals during the periods of treatment.

Results. The numbers of blood smears found positive for *P. malariae* are given in table 2 where the data are broken down by school, group and date of examination. The total number of positive slides found in the control groups during the time the experimental groups were being treated is 58, while none were found in the treated groups. These 58 positive slides represent 35 individuals, since a number of individuals were found positive more than once. Regardless of whether the total number of positives or the more conservative figure on the number of infected individuals is used, there is no doubt that metachloridine has a marked effect on this species of parasite. Since blood smears were only taken from those attending school, anyone absent because of clinical malaria would not show up in these data. The incidence shown for the control group then may be lower than it actually was.

The same type of data for *P. falciparum* is given in table 3. Ninety-three positive slides were found in the control groups during the treatment period while only 37 were found in the experimental groups during the periods they were treated. If this is put on the basis of infected individuals there were 60 in the controls and 24 in the treated group. These differences are highly significant statistically.

The data regarding *P. vivax* are not tabulated since only 11 positive slides were found in the control groups during the treatment periods. Only 1 positive slide was found in the treated group, and it is possible that this person avoided taking the drug. This reduction would be expected from the results of Pullman already referred to.

DISCUSSION

The conditions for testing an antimalarial drug, as far as the occurrence of malaria is concerned, were favorable during the period of the trial, as can be judged from the incidence in the controls (tables 2 and 3). During the first few months the incidence was rather uniform. During July the incidence increased markedly and was still high 50 days after the trial was terminated. This marked rise in incidence in the controls increases the significance of the absence or low incidence of malaria in the treated groups.

The unquestionable effect of metachloridine on *P. malariae*, as illustrated in table 2, indicates the desirability of determining the minimum effective dose,

and leads to speculation on the exact action the drug may be having on this infection. The minimum effective dose would be relatively small if *P. malariae* proved to be as sensitive to the action of metachloridine as is *P. gallinaceum*, sporozoite-induced infections of which were suppressed by as little as 0.2 mg. of drug per kg. daily. On this basis, 12 mg. of metachloridine daily should be suppressive to *P. malariae* in an individual weighing 60 kgs. If it were as sensi-

TABLE 2

Incidence of blood smears positive for P. malariae in school children treated with metachloridine

DATE OF EXAMINATION	UNTREATED CONTROLS						TREATED*					
	School				Total Positives	New Positives	School				Total Positives	New Positives
	B	P	E	F†			B	P	E	F		
pre-treatment.....	0	2	5	2	9	9	2	2	1	3	8 0	8 0
February 26-March 12.	2	1	5	0	8	5	0	0	0	0	0 0	0 0
March 18-April 2....	0	2	1	3	6	2	0	0	0	0	0	0 0
April 9-April 16....	1	0	2	1	4	1	0	0	0	0	0	
Post holiday.....	1	0	1	1	3	1	0	0	0	1	1	1
July 4.....		9	5	6	20	17		0	0	0	0	0
July 25.....		8	5	7	20	10		0	0	0	0	0
50 days post treat- ment.....		5	10	8	23	8		2	4	4	10	10
Totals during treat- ment period.....	3	20	18	17	58	35	0	0	0	0	0	0

*Treatment: 1 gm./week, adult basis, February 4-April 17, 1946 and May 6-May 30, 1946
2 gm./week, adult basis, May 30-July 26, 1946

†B = Blairmont

P = Providence

E = Edinburgh

F = Friends

- tive as *P. vivax* about 0.25 gm. per week would be required for suppression (Pullman, loc. cit.). The smallest doses used in the trials reported here were in the younger children (table 1) where the dosage was 200 to 300 mg. per week (12-18 mg./kg./week) during the latter period and would be half this during the earlier period of treatment. Eight blood positives were found in untreated controls in this same age group.

It cannot be determined from our data whether metachloridine completely

prevented or eradicated *P. malariae* infections or whether it produced long-term suppression. Fifty days after treatment was terminated there were 23 positive slides from the control groups and only 10 from the treated groups. However, only 8 of the 23 positives in the control group represented new cases. This numerical coincidence in both groups suggests that the ten cases of the treated group did not represent relapses but new infections. The precise effect of metachloridine on *P. malariae* can only be answered by using experimentally-induced infections in individuals that are under observation for extensive periods during which there is no exposure to reinfection.

TABLE 3

Incidence of Blood Smears Positive for P. falciparum in School Children Treated with Metachloridine

DATE OF EXAMINATION	UNTREATED CONTROLS						TREATED*					
	Schools†				Total Positives	New Positives	Schools				Total Positives	New Positives
	B	P	E	F			B	P	E	F		
pre-treatment.....	0	1	4	3	8	8	1	3	3	0	7	7
February 26-												
March 12.....	5	1	7	1	14	10	2	0	2	0	4	4
March 18-April 2....	4	1	2	5	12	9	6	0	1	2	9	7
April 9-April 16....	2	2	3	0	7	5	3	1	2	1	7	5
Post holiday.....	0	2	8	3	13	9	1	4	8	3	16	9
July 4.....		5	13	8	26	18		2	1	2	5	1
July 25.....		16	10	8	34	18		7	5	0	12	7
50 days post treat- ment.....		13	10	8	31	8		9	8	7	24	14
Totals during treat- ment period.....	11	25	35	22	93	60	11	10	11	5	37	24

* Treatment: 1 gm./week, adult basis, February 4-April 17, 1946 and May 6-May 30, 1946
2 gm./week, adult basis, May 30-July 26, 1946

† See Table 2 for key to school names.

The incomplete suppression of parasitemia with *P. falciparum* is disturbing, because of the importance of this infection in many areas. The increase in dosage from 1 gram per week to 2 grams per week, adult basis, mentioned previously was an unsuccessful attempt to bring this species under control, and the data suggest a greater reduction in the incidence of parasitemia during the period of increased dosage. When the dosage was 1 gram per week, adult basis, 33 blood smears positive for *P. falciparum* were found in the control groups while 20 were found in the treated group. When the dosage was 2 grams per week the numbers of positive slides were 60 and 17 respectively. The reduction in incidence of parasitemia in the treated group as compared with the control groups was 40 per cent during treatment with 1 gram and 72 per cent during treatment with 2 grams. The method of dividing the doses resulted in some variation in the proportional dose received by different children, thus permitting some comparison to be made between the size of the dose received and the occurrence of parasitemia (table 4).

The data in table 4 cover the period during which the larger dosage schedule was used (May 30 to July 26, 1946). This analysis also suggests an increased effectiveness of larger doses, although there was still an occasional positive in individuals receiving as much as 2 grams of drug per week. This is in line with the results of Packer (1947), who found that one gram of metachloridine weekly did not prevent experimental sporozoite-induced infections of *P. falciparum*, while 2 grams had a definite but variable effect on these infections. It seems possible that only slightly larger doses might completely suppress *P. falciparum*.

From a theoretical, and perhaps from an epidemiological point of view, the presence of parasites in the blood may be of importance, but if the drug completely suppressed clinical manifestations, it could be considered effective from the practical viewpoint of an individual who is only interested in staying well.

TABLE 4

Relationship Between Metachloridine Dosage and Parasitemia with P. falciparum

DOSAGE GRAMS*	TREATED GROUP		CONTROL GROUP	
	Proportion of Individuals Positive	% Individuals Positive	Proportion of Individuals Positive	% Individuals Positive
<1.0	4/12	33	7/11	63
1.0-1.5	3/33	9	11/36	31
1.6-1.9	4/38	10	9/42	21
2.0	1/31	3	9/31	29
>2.0	2/32	6	11/30	37

* Grams drug per week converted to adult basis.

It is perfectly possible that metachloridine at the doses used suppressed infection with *P. falciparum* below pyrogenic levels, even though it did not always reduce the parasitemia below the microscopically visible level. Unfortunately, there are no good data regarding this matter, since it was not possible to check the absentees daily to determine the cause of their absence. In March there was a total of 368 days of absence in the control groups and 290 days in the treated group. In July this was 182 as compared with 142. This is suggestive of an improvement in attendance due to the use of metachloridine, but it is of extremely little value without information on the cause of each absence. As a matter of fact, the improvement might have been due exclusively to the suppression of malarial malaria. For what it is worth as subjective information, it is interesting that the teachers in the schools were all firmly convinced that there was a marked improvement in the health and mental attitude of the children in the treated groups, as compared with the untreated groups. An analysis of the crude quantitative data indicates that there were proportionately about as many slides with numerous parasites in the treated group as in the control group. Also, there were proportionately about as many with gametocytes in each group.

It is important to keep in mind the effect that such a partially active drug might have on incidence data, if smears are only made routinely on individuals in attendance at school. The apparent incidence in the untreated group would

be lower than the real incidence due to the absence of children sick with malaria. On the other hand, treatment would suppress some clinical malaria while not rendering their blood negative, which might increase the apparent incidence in the treated group.

The partial reduction in parasitemia with *P. falciparum* observed in these trials in school children, was also seen in a group consisting chiefly of adults in Kwakwani, an inland mining village. This trial is not described in detail, since the incidence in the control groups dropped almost to zero during the experimental period. About 160 individuals were treated with 0.5 gram metachloridine once each week for three months, followed by 0.5 gram twice each week for three months. Blood smears were made routinely from everyone twice each month and at other times from anyone with an elevated temperature or signs of clinical malaria. Twelve smears positive with *P. falciparum* were found in this group or 7.5 per hundred individuals. During the trial about 85 untreated individuals were examined in the same way. These constituted the control group. Fourteen slides positive with *P. falciparum* were found or 16.5 per hundred individuals. As is seen, the difference between the incidence of *P. falciparum* is about of the same magnitude as in the groups of school children. Other species of malaria parasites were found too infrequently in the control groups at Kwakwani to justify any comments.

While the data recorded here and those available from other sources (Survey of Antimalarials) do not permit an exact quantitative estimate of the relative activity of metachloridine against the three species of malaria parasites of man, it is quite evident that *P. falciparum* is least susceptible to the action of this drug. In fact, it is the only species of parasite of bird or man tried to date that is not highly sensitive to this drug, and illustrates the unpredictability of drug action from one species of parasite to another, which is coming to be accepted as axiomatic among malariologists.

Limited conclusions can be drawn regarding the toxicity of metachloridine. Not a single toxic reaction was noted in any of the trials in British Guiana. The numbers of individuals involved and the dosages and extent of treatment are shown in the following tabulation:

100 School children (New Amsterdam):	1 gm. (adult basis) per week for 4 months
150 School children (New Amsterdam):	1 gm. (adult basis) per week for 4 months followed by 2 gm. (adult basis) per week for 2 months
150 all ages, mostly adults (Kwakwani):	0.5 gm. per week for 3 months followed by 1.0 gm. per week for 3 months
250 + adults (mental hospital):	0.1 gm. per week for 3 months fol- lowed by 1.0 gm. per week for 3 months

The trials in the mental hospital were designed to determine the effect of smaller doses, but are not discussed further here since only 6 positive blood smears were discovered in the controls during the 6 month period of the trial. One positive for *P. falciparum* was found in the treated group.

SUMMARY AND CONCLUSIONS

Field trials in British Guiana in 1946 showed that metachloridine at doses of 1 to 2 grams weekly, divided into 2, 3 or 5 parts, completely suppressed natural infections of *P. malariae* and partially suppressed parasitemia with *P. falciparum*. Limited data indicate good suppression of *P. vivax*. No toxicity was seen during the treatment period of 6 months. Lower doses should be tried to determine the minimum effective dose for the suppression of *P. malariae* and higher doses should be tried for the same purpose in infections with *P. falciparum*. The ability of metachloridine to suppress clinical symptoms of infections with *P. falciparum* should be determined.

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FURTHER NOTE ON THE CLASSIFICATION OF VIBRIOS OF THE 1945 CHOLERA EPIDEMIC IN CHUNGKING

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In studies of the cholera epidemic in Chungking in 1945 (1), cultures of vibrios derived from several patients were classified as of the Ogawa type in one laboratory. The same strains were sent to Dr. William Burrows of the University of Chicago, who reports different results (2). He has recently proposed the classification of *Vibrio comma* on a serologic basis, but substituting the eponymic or place-name classification with a preferable one of capital letters to indicate the antigenic structure (3). According to his studies, three strains were classified as type AB or of the Ogawa type; two were type AC or the Inaba type; two were type ABC corresponding to the Hikojima type, and two contained antigen B but lacked A. The last mentioned, according to Burrows' scheme of classification, would not belong to 0 subgroup 1 and presumably are not cholera vibrios. Strains of the various types, as classified, showed no orderly pattern of resistance to streptomycin. Certain strains of each type were sensitive to streptomycin and others were highly resistant.

One would expect to encounter only a single type of cholera vibrio in an epidemic among persons in the community from which the patients studied were drawn. Localized epidemics of typhoid, for example, are often caused by a single serologic type of *E. typhosa*. However, in outbreaks of influenza or of pneumococcal pneumonia one or more types of the respective causative agents may participate in varying proportion and combination.

In epidemics of cholera, both circumstances apparently occur; namely, either a single type strain or different serologic types of *V. comma* are rampant (4). Questions arise as to (a) whether epidemics begin from the distribution of a single type of *V. comma* and other types appear as a result of bacterial dissociation or type transformation, or if epidemics are caused by one or more stable types of vibrio; (b) if the vibrios devoid of A antigen and presumably not *V. comma* may cause clinical cholera as such, or if they represent dissociant culture phase forms of pathogenic vibrios induced by growth outside of the body.

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THE PRESENT STATUS OF HOOKWORM INFECTION IN FLORIDA

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Hookworm infection has rightly been considered the foremost parasitic problem in Florida. These parasites, introduced into the State from Africa, encountered the three requisites necessary for their propagation; equitable temperature throughout the year, ample rainfall, and sandy, well drained soil. These factors in an area where a large part of the rural population lived under poor economic conditions provided ideal opportunity for establishing a high incidence and intensity of infection.

HISTORICAL BACKGROUND

Hookworm infection was recognized as an important medical problem in Florida as early as 1903 (3). From January of that year until 1909, an active anti-hookworm campaign was carried to the physicians and teachers of the State by the Board of Health. Accurate diagnosis by microscopic examination of fecal material was begun in 1909. The first survey was made in 1910, just prior to the one conducted in the other southeastern states by the Rockefeller Sanitary Commission (15), later known as the International Health Board of the Rockefeller Foundation. This first Florida survey showed that 58.1 percent of the 6155 persons examined had hookworm infection (3).

In 1926, the International Health Board of the Rockefeller Foundation (13) conducted a major hookworm survey in cooperation with the State Board of Health. This investigation revealed an average hookworm incidence of 56.2 percent, varying in different sections of the State from 26.5 to 97.0 percent. The average incidence is not significantly different from that reported by the Florida Board of Health in 1910. The intensity of infection was determined in this second survey.

When the hookworm problem was first studied it was assumed that infection and disease were synonymous. It was soon noted that some infected individuals appeared in good health while others exhibited definite symptoms of disease. Microscopic examination of the fecal material showed that the clinical manifestations were roughly proportional to the concentration of hookworm eggs. Darling (8) had observed during his anti-hookworm campaign in Brazil that a high incidence of infection remained after treatment although the degree of infection was light. A scientific method of measuring this intensity was reported by Stoll in 1923 (17). Many modifications, notably the one by Stoll and Hausheer (18) have been based on the original Stoll dilution egg count technic.

The significance of the varying intensities of infection was established by Smillie and Augustine in 1926 (16). By means of the Stoll count a number of Alabama children who had hookworm infection were divided into intensity

groups. The divisions, based on the number of hookworm eggs per cubic centimeter of fecal material, were designated as very light (200-699 eggs/cc.), light (700-2599 eggs/cc.), moderate (2600-12,599 eggs/cc.) and heavy (12,600-over eggs/cc.). Each child was given a careful physical examination. Treatment was administered to those who harbored hookworms. A second examination, made three months after treatment, showed that the physical improvement of the children who had been freed from their hookworms was proportional to the intensity of the original infection. The children who had had heavy or moderate infections showed marked improvement, those in whom the infection had been of light intensity showed minor gains, while no measurable benefit could be observed in the children whose original infection had been of very light intensity. It was also noted that the children in whom these heavier infections had not been cured but had been reduced to the very light intensity level showed equal improvement with those in whom a cure had been established.

In view of these observations the very light infections were considered of no clinical significance and those of light intensity of doubtful moment. Only the moderate and heavy infections were believed to be of unquestionable clinical importance. Their intensity groupings, corroborated by many, have been accepted as the standard for the evaluation of the significance of the various intensities of hookworm infection. Andrews (2) while accepting the general classification, believed the basis for division between the significant and non-significant groups too conservative for public health field work.

Kerr (13) applying the Smillie and Augustine values in the 1926 survey which he conducted in Florida found that the most significant infections, that is, those of moderate or heavy intensity, comprised 8.3 percent of the total number examined.

The third important hookworm survey was made under the direction of the Vanderbilt group (14) in 1937-1938. Examination of 29,064 specimens showed a 34.8 percent incidence of infection. The moderate and heavy intensity groups, those which include most of the cases of hookworm disease, accounted for 11.5 percent of the whole.

Many thousands of hookworm examinations have been made every year by the State Board of Health. With the exception of the surveys mentioned the intensity of infection has not been considered. Repeated surveys have shown that the treatment programs have not materially affected the general hookworm level for several years. It seemed desirable to approach the problem on the basis of intensity of infection to determine if this static condition were apparent or real. Such information would show if there has been a trend toward lighter infections. In addition, follow-up work could be concentrated on the groups showing the most significant infections. Treatment of those individuals with improvement of their environment, would reduce a community focus of infection thereby bringing far greater returns for effort expended.

As an aid to a more effective hookworm control program the intensity of all positive hookworm infections are now reported from the central and from two of the branch laboratories of the Board of Health.

As no quantitative evaluation of the problem had been reported since 1938, our findings to date are presented.

METHODS USED IN THE VARIOUS STUDIES

The 1910-14 survey was made by direct examination of a single saline preparation. The Caldwell and Caldwell (5) technic was employed in 1926 and the Stoll count (18) in the 1937-38 survey. In 1942 the Jacksonville Laboratory of the Florida State Board of Health adopted the more efficient Faust ZnSO_4 (9) concentration technic as the single examination for all fecal specimens. A major value of this method is that protozoan cysts are preserved so that cases of amebiasis in the cyst producing stage are revealed. A possible disadvantage is that it detects many hookworm infections of such light intensity that they are clinically unimportant. The need of distinguishing between these light infections and the heavier ones which are actually or potentially significant has been recognized by many workers for many years (Andrews (1); Chandler (6); Cort (7)).

TABLE 1
Values of intensity of infection

INTENSITY	ACCEPTED VALUES Eggs/cc.	ACTUAL ZnSO_4 COUNTS Eggs/cover glass
Very Light.....	200-699	1-40
Light.....	700-2599	41-150
Moderate.....	2600-12,599	151-700
Heavy.....	12,600-over	701-over

By such means the present hookworm problem in Florida has been studied.

The value of the Stoll egg count for estimating the intensity of infection is well established but is impractical for routine use in a large public health laboratory. Several more simple methods have been devised (5, 11) by which the intensity of hookworm infection can be estimated. As none of these served our needs a simple technic based on egg counts per cover slip preparation of the standard ZnSO_4 concentration method already in use was developed in the Jacksonville laboratory (10). Parallel egg counts made by the Stoll and ZnSO_4 technics established a ratio by means of which the ZnSO_4 count could be converted to a Stoll count equivalent, from which the number of eggs per cubic centimeter of fecal material could be derived. These actual counts, together with their equivalent number of eggs per cubic centimeter of fecal material are shown. The values for the estimation of intensity of hookworm infection used in this study were a conservative approximation of those established by Smillie and Augustine (16).

RESULTS

Specimens from white and colored schools were evaluated separately, but such a division was not feasible in the general diagnostic work.

The following tables give the actual numbers and percents of the positive and negative specimens and the intensity rating of those found positive for hookworms. The charts show only the intensity distribution of the positive cases.

Table 2 and Chart I, give hookworm data for several white and colored schools representing 12 counties.

The wide variation in the incidence of infection of the various groups is noteworthy. The incidence is still high but as Chart I shows, the sum of the moderate and heavy infections exceed 10 percent of the positives in only two schools.

TABLE 2
Incidence and intensity of hookworm infection found in school surveys

COUNTY	TOTAL	NEGATIVE		POSITIVE		INTENSITY DISTRIBUTION			
		No.	Per cent	No.	Per cent	Very Light	Light	Moderate	Heavy
						Per cent	Per cent	Per cent	Per cent
White Children									
Alachua.....	2317	1729	74.7	588	25.3	20.3	3.1	1.7	.2
Gadsden.....	289	130	44.9	159	55.1	37.3	11.5	4.6	1.7
Gilchrist.....	569	227	39.9	342	60.1	46.8	10.0	2.3	1.0
Hamilton.....	2318	1148	49.6	1170	50.4	37.9	7.6	4.3	.6
Jackson.....	316	111	35.2	205	64.8	53.8	6.0	3.8	1.2
Lake.....	283	223	78.8	60	21.2	15.2	4.6	1.4	0
Leon.....	660	541	81.9	119	18.1	15.8	1.6	.7	0
Nassau.....	204	73	35.7	131	64.3	40.2	13.4	6.8	3.9
Polk.....	834	456	54.6	378	45.4	31.8	9.2	3.8	.6
Taylor.....	186	129	69.4	57	30.6	23.2	5.3	2.1	0
Total.....	8017	4767	59.5	3250	40.5	30.4	6.2	2.9	1.0
Colored Children									
Bay.....	129	126	97.6	3	2.4	2.4	0	0	0
Gilchrist.....	77	68	88.3	9	11.7	11.7	0	0	0
Hamilton.....	827	715	86.5	112	13.5	11.8	1.3	.2	.2
Lake.....	54	49	90.7	5	9.3	7.4	1.8	0	0
Sumter.....	177	136	76.9	41	23.1	21.4	1.7	0	0
Total.....	1264	1094	86.7	170	13.3	11.9	1.1	.15	.15

Both incidence and intensity of infection are markedly less in the colored than in the white children. This is in accordance with other observations in Florida (14) and elsewhere (12).

Table 3 and Chart II present data which show the influence of various factors on the distribution of hookworm infection.

Section A. The greatest incidence and intensity of hookworm infection in this series fall in the 10-14 age group. A marked diminution appears after the 20th year. These observations place the highest infection rate at a slightly

Chart I

Intensity Grouping of the Positive Hookworm Cases

Very Light ▨ Light ▩ Moderate ▪ Heavy ▣ Shown in Table I.

White School Surveys.

County	0%	20%	40%	60%	80%	100%	V.L.	Actual L.	Percent M.	H.
Alachua							79.8	12.3	6.9	1.0
Gadsden							67.9	20.8	8.2	3.1
Gilchrist							77.8	16.7	3.8	1.7
Hamilton							75.3	14.9	8.5	1.3
Jackson							82.9	9.3	5.9	1.9
Lake							71.7	21.7	6.6	0
Leon							87.4	8.4	4.2	0
Nassau							62.7	20.6	10.6	6.1
Polk							70.1	20.2	8.4	1.3
Taylor							75.5	17.5	7.0	0
Total							74.8	15.2	7.3	2.7

Colored School Surveys.

	0%	20%	40%	60%	80%	100%				
Bay							100			
Gilchrist							100			
Hamilton							86.7	9.9	1.7	1.7
Lake							80.0	20.0		
Sumter							92.7	7.3		
Total							88.8	8.8	1.2	1.2

TABLE 3

Incidence and intensity of hookworm infection distribution according to age, sex, color

SECTION A, AGE GROUPING, GENERAL POPULATION	TOTAL	NEGATIVE		POSITIVE		INTENSITY DISTRIBUTION			
		No.	Per cent	No.	Per cent	Very light	Light	Moderate	Heavy
						Per cent	Per cent	Per cent	Per cent
0-9	2702	1923	71.2	779	28.8	20.8	4.5	2.3	1.2
10-14	1293	747	57.8	546	42.2	31.0	6.8	2.3	2.1
15-19	429	286	66.7	143	33.3	25.2	4.8	3.0	.3
20-29	342	257	75.1	85	24.9	22.2	1.5	1.2	0
30-39	281	250	88.9	31	11.1	9.3	1.4	.4	0
40-49	171	155	90.7	16	9.3	8.2	1.1	0	0
50-over	161	155	96.3	6	3.7	3.7	0	0	0
SECTION B, SEX GROUPING, WHITE CHILDREN									
Girls	1009	547	54.3	462	45.7	34.8	7.0	3.3	.6
Boys	1025	500	48.8	525	51.2	37.9	8.9	3.4	1.0
SECTION C, COLOR GROUP- ING, ADULT MALES									
White	371	251	67.8	120	32.2	29.4	2.4	.2	.2
Colored	246	221	89.8	25	10.2	10.2	0	0	0

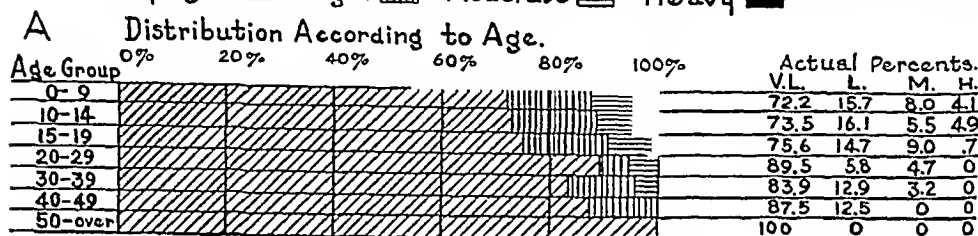
younger level than those of Leathers *et al.* (14), but are in general accord with their findings and those of other workers.

Section B. In grouping 2034 white children according to sex, the girls show slightly lower values but no practical difference is noted in either incidence or intensity of hookworm infection.

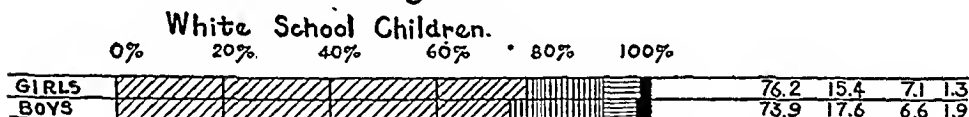
Section C. A group of adult males were examined for possible hookworm infection. As would be expected from Table I and Chart I both the incidence and intensity of the infection are greater in the white than in the colored group. Even in the white group the values are decidedly below those of the children. This is compatible with the data shown in Table II Section A which demonstrates the marked diminution of hookworm infection in adults.

Chart II

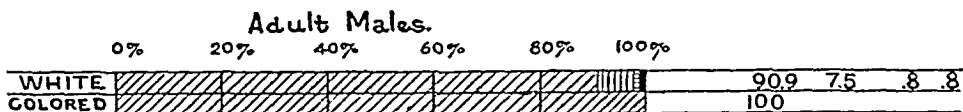
Intensity Grouping of the Positive Hookworm Cases
 Very Light ▨ Light ▩ Moderate ▪ Heavy ■ Shown in Table II.



B Distribution According to Sex.



C Distribution According to Color.



The work of Leathers *et al.* (14) showed that the greatest hookworm incidence in Florida was in the western section of the State although the greatest average intensity was in the northeastern section. To determine whether or not the present distribution follows this pattern the findings from all the diagnostic specimens submitted during the period of November 19, 1946 through January 8, 1947, were tabulated according to geographical distribution. When 100 or more specimens were received from a county the results were so designated. Those counties submitting less than 100 specimens during this test period were grouped and so included in their respective areas.

The geographic divisions used were those established by the Vanderbilt group (14) so that any changes in hookworm infection that may have occurred could be compared.

Table 4 and Chart III give the present incidence and intensity grouping⁷ of hookworm infection in the State as shown by the specimens submitted during the test period.

Although our studies are somewhat less extensive than those made in 1937-1938, it is immediately evident that the general pattern of distribution of hookworm infection is similar. The western section still has the highest incidence

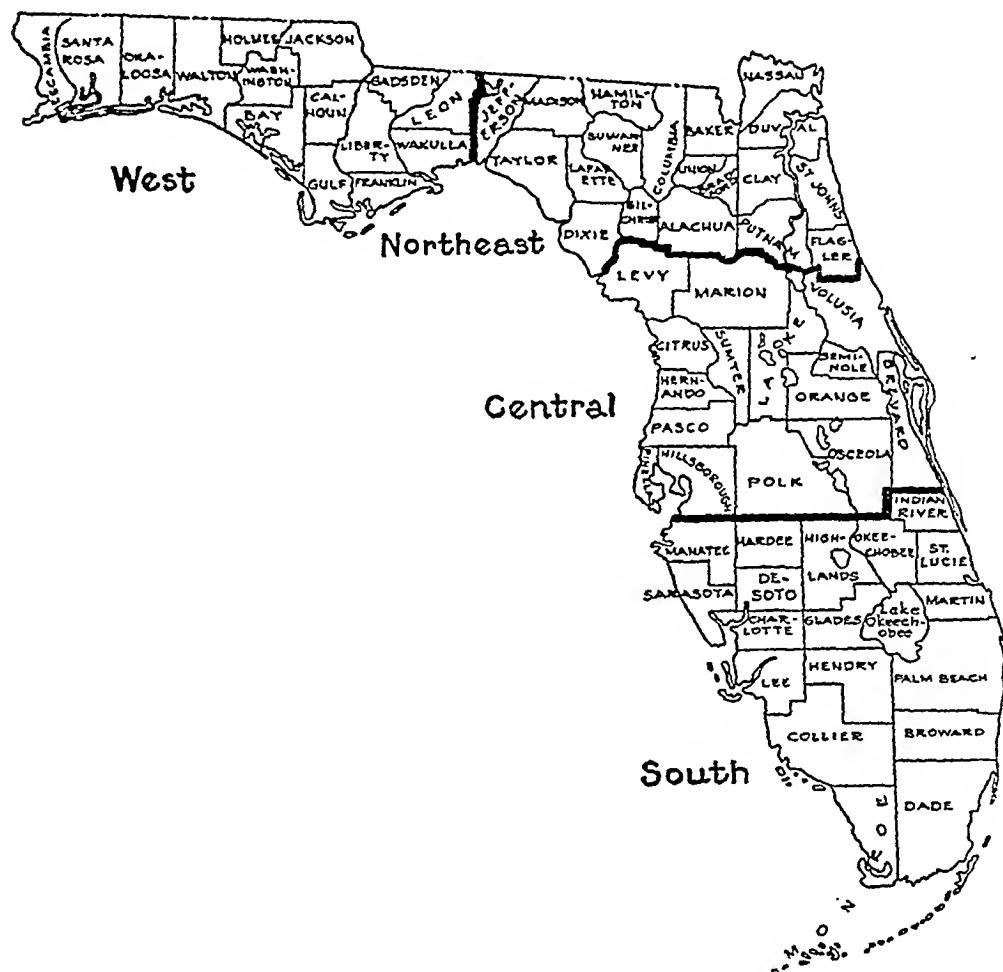


FIG. 1. STATE OF FLORIDA

Geographic Divisions used to show distribution of Hookworm Infection.

of infection, where 51.6 percent of the examinations are positive, in contrast to 25.6, 19.1 and 18.7 percent in the northeast, central and south sections respectively.

Our data show a slight increase in incidence of infection in the western section over that found in the Vanderbilt survey. That this is an apparent rather than a real increase is explained below. A marked diminution is noted throughout the remainder of the state compared to the values established in 1937-38 which

TABLE 4
Incidence and Intensity of Hookworm Infection Geographic Distribution

Incidence and Intensity of Hookworm Infection Geographically									
SECTIONS BY COUNTIES	TOTAL	NEGATIVE		POSITIVE		VERY LIGHT	LIGHT	MODERATE	HEAVY
		No.	Per cent	No.	Per cent	Per cent	Per cent	Per cent	Per cent
West									
Bay.....	398	201	50.6	197	49.4	28.9	9.3	5.2	6.0
Gadsden.....	327	167	51.0	160	49.0	33.4	10.5	3.6	1.5
Holmes.....	243	68	27.9	175	72.1	44.4	13.1	9.0	5.6
Jackson.....	310	168	54.2	142	45.8	36.8	4.5	2.9	1.6
Washington.....	176	86	48.9	90	51.1	38.1	8.5	3.4	1.1
Others.....	202	110	54.5	92	45.5	35.2	4.9	4.4	1.0
Total.....	1656	800	48.4	856	51.6	35.3	8.6	4.7	3.0
Northeast									
Alachua.....	157	131	83.5	26	16.5	10.2	3.9	1.2	1.2
Duval.....	1330	1167	87.8	163	12.2	10.9	.8	.5	0
Jefferson.....	147	91	61.9	56	38.1	30.7	6.7	0	.7
Lafayette.....	107	33	30.9	74	69.1	46.8	11.2	9.3	1.8
Taylor.....	668	384	57.5	284	42.5	35.9	4.7	1.3	.6
Others.....	613	441	71.9	172	28.1	22.1	3.8	1.8	.4
Total.....	3022	2247	74.4	775	25.6	20.9	3.1	1.3	.3
Central									
Lake.....	329	240	72.9	89	27.1	18.6	4.6	2.4	1.5
Marion.....	120	93	77.5	27	22.5	18.3	3.3	.9	0
Orange.....	332	298	89.8	34	10.2	7.2	1.5	1.2	.3
Polk.....	212	168	79.3	45	20.7	17.5	1.4	1.4	.4
Volusia.....	313	265	84.7	48	15.3	12.8	1.0	1.5	0
Others.....	337	264	78.4	73	21.6	16.4	4.1	.8	.3
Total.....	1643	1328	80.9	315	19.1	14.6	2.7	1.4	.4
South									
Martin.....	245	214	87.3	31	12.7	11.6	1.1	0	0
Others.....	374	289	77.3	85	22.7	14.8	4.8	2.6	.5
Total.....	619	503	81.3	116	18.7	13.5	3.3	1.6	.3
Grand Total.....	6940	4878	70.3	2062	29.7	22.2	4.4	2.1	1.0

showed 49.2, 38.0, 25.2 and 23.3 percent respectively for the western, north-eastern, central, and southern sections. In the present series the greatest intensity of infection is in this western area (Chart III). The sum of the moderate and heavy groups, i.e., the infections which most consistently produce hook-

worm disease, is 15.1 percent of those found positive in the western area, in contrast to 6.5, 10.2, and 10.8 percent in the northeast, central and south sections respectively.

Chart III Intensity Grouping of the Positive Hookworm Cases
Sections Very Light █ Light █ Moderate █ Heavy █ Shown in Table III.

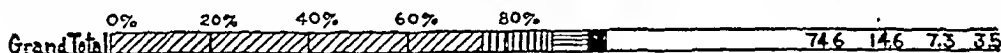
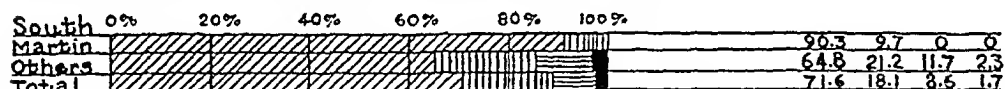
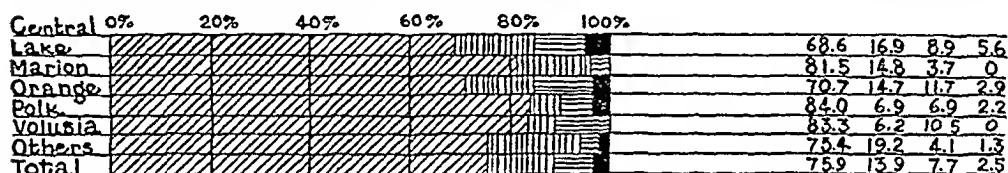
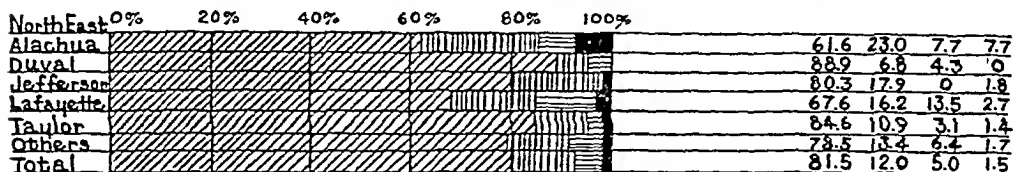
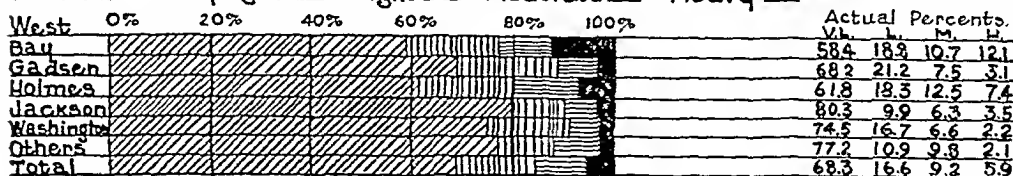


TABLE 5

Summary of the Present Investigation of the Incidence and Intensity of Hookworm Infection in the State of Florida

TOTAL	NEGATIVE		POSITIVE		VERY LIGHT	LIGHT	MODERATE	HEAVY
	No.	Per cent	No.	Per cent	Per cent	Per cent	Per cent	Per cent
16838	11211	66.5	5627	33.5	25.32	4.9	2.3	.98
							33.5	
								3.28

The examination of 16,838 specimens in the present study show the following distribution of incidence and intensity of hookworm infection.

The 33.5 percent incidence is only 1.3 percent below that reported in 1937-'38. This reduction is in reality greater because the more sensitive technic now used detects lighter infections, thereby increasing the number of cases found. The intensity of infections shows a marked actual drop as only 3.28 percent of the

total number examined fall in the moderate or heavy group in contrast to 8.3 percent and 11.5 percent demonstrated in the 1926 and the 1937-'38 surveys.

DISCUSSION

The Bureau of Laboratories of the State Board of Health has offered continuous service since 1909 to determine the incidence of hookworm infection throughout the state. Approximately, 70,000 fecal examinations per annum have been made for the past several years. Many thousands of treatments have been administered by the county health units and the local physicians. In addition, information has been continuously disseminated by means of printed matter and educational programs. Particular emphasis has been placed on the ultimate control of the infection through proper sanitation. The present study was undertaken to determine if all of these factors, together with the generally improved economic status of the citizens has materially reduced hookworm infection throughout the state.

Although approximately 40 percent of the white children examined have hookworm infection, less than 4 percent of the total or 10 percent of those found positive show infections of sufficient intensity to place them in the Moderate or Heavy groups. According to Smillie and Augustine these account for the infections of unquestioned clinical significance.

The data shown in Table II Chart II indicate that this infection is one of childhood. This is practically true in Florida, not because of a special immunity of adults but rather because of their improved personal hygiene. The work of Darling (8) in the Federated Malay States showed the hookworm infection rate in adults even slightly higher than that in children.

Very little difference in infection rate is shown between the sexes. This is not universally true but again depends upon the personal habits of the groups compared.

A low rate of infection is shown in the group of adult males surveyed. A marked difference is evident when division is made according to race with lower values seen in the colored men. A similar variation is demonstrated between the white and colored children in the school survey (Chart I). This appears to be a true racial difference in susceptibility to hookworm infection that is observed wherever surveys are made.

The examination of 16,838 specimens in the present study show a hookworm incidence of 33.5 percent. Only 3.28 percent of the total have infections of moderate and heavy intensity in contrast to 11.5 percent demonstrated in the 1937-'38 survey. This reduction of intensity of infection has been accomplished by improved sanitation and yearly treatment of many individuals. This latter procedure when persistently followed, as has been done in Florida, eliminates most of the clinical cases. Unless proper sanitation is provided, reinfection occurs and the infections persist. While this marked reduction is gratifying, there is still too much hookworm disease in Florida to warrant complacency.

Heretofore the only report on hookworm infection given by the State Board of Health was on the presence or absence of eggs in the specimens submitted. A

new approach to the problem is made possible by the present procedure of reporting all cases according to the intensity of infection; very light, light, moderate, or heavy. This added information will enable the school authorities, the local physicians, and the county health units to more adequately evaluate the hookworm problem in their own communities. A more effective anti-hookworm program should result which could ultimately bring about the eradication of hookworm disease from Florida.

CONCLUSIONS

1. The need of evaluation of intensity of hookworm infection is presented.
2. A simple and sufficiently accurate method for determining the intensity of infection is briefly described.
3. A study of 8017 white school children shows a marked variation between the counties in both incidence and intensity of infection. Approximately 40 percent of the white children have hookworm infection but only 3.9 percent of the whole number or 10 percent of the positive cases are classed in the moderate and heavy intensity groups.
4. A very low incidence and intensity of hookworm infection are shown in a group of 1264 colored children studied.
5. Hookworm infection is most marked during school age. A sharp diminution in both incidence and intensity of infection is shown about the twentieth year.
6. Both incidence and intensity of hookworm infection are greater in the western section than in any other area of the State.
7. While there has been marked diminution in the intensity of hookworm infection in the last 10 years this disease is still an important public health problem in the State.
8. It is believed that the present method of reporting all hookworm cases according to the intensity of infection will be of major help in evaluating the problem. A more effective anti-hookworm program can be pursued which should lead to the ultimate eradication of the disease.

ACKNOWLEDGMENTS

Appreciation is extended to Dr. A. V. Hardy, Director of Laboratories of the Florida State Board of Health for his interest during the progress of the work and his helpful criticism of the manuscript.

Acknowledgment is made to Miss Jeanette Washburn for her excellent service in making the charts.

A special debt is owed Mr. Mun Quan, Junior Parasitologist, and the technicians whose efficient work made this study possible.

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LIBERIA, SCENE OF NEW INTERNATIONAL INSTITUTE FOR TROPICAL MEDICINE

The establishment of a tropical disease research center in Liberia, open to all medical students and scientists without restriction as to nationality, race or creed, has been announced by Dr. Thomas T. Mackie, president of the American Foundation for Tropical Medicine.

"Over 50 per cent of the world's population lives in tropical areas, subject to many little understood diseases, yet at the time of Pearl Harbor, we found there were only 24 civilian doctors in the entire United States with background training in tropical medicine," Dr. Mackie said, pointing out the immediate need for extensive research in this field.

Liberia, the African Republic founded just 100 years ago by freed American slaves, offers ideal conditions to study the three factors which at present keep the peoples of the tropics in virtual bondage, Dr. Mackie continued. These factors are 1) ineffective agriculture with poor crop yield, 2) prevalence of highly endemic diseases of domestic animals, which together make for almost universal malnutrition; and 3) uncontrolled human diseases which lead to high infant mortality rates and insufficient population. These same diseases cause the high adult morbidity and mortality.

The \$250,000 research Institute, to be erected outside the capital city of Monrovia this year, is a gift from Harvey S. Firestone, Jr., as a memorial to his late father. It will be located on a 100 acre tract adjoining Roberts Field, international airport of West Africa. This land, together with 600 additional acres of farm and grazing land for agricultural investigations, is a tax-free gift from the Liberian Government. In a letter to Dr. Mackie, following his visit to Liberia last fall, His Excellency the Honorable William V. S. Tubman, President of the Republic wrote: "We look forward to the benefits that we hope will be derived from the project envisaged by the Institute. You may be further assured that the project will have the full support and the cooperation of the Liberian Government."

The Liberian Institute of the American Foundation for Tropical Medicine is expected to become an international center for research into the medical and related problems of the tropics. Open to all students and scientists interested in these problems, it will offer unparalleled opportunities to study both human and animal diseases in their native habitat. All findings resulting from these studies will be made available freely to other students and researchers in all parts of the world. It is hoped that the opportunity for free exchange of information among individual scientists working together on related problems will provide the mechanism for the development of new techniques and their rapid evaluation in the treatment and control of disease.

Liberia's modern seacoast capital and primitive mountain villages, is just two days from New York by plane. In an area a little bigger than the state of Ohio, its abundant natural resources are relatively undeveloped due to wide-spread

malaria, sleeping sickness, and other diseases. Domestic animals are virtually unknown in many areas as they too are victims of the parasites and fevers which attack the people. In spite of these handicaps, Liberia is one of the few countries of the world with a balanced budget and has maintained her position as the one free republic in Africa against tremendous odds. "The close friendly relations which exist between our country and this Republic," Dr. Mackie pointed out, "constitute an important safeguard against complications, all too often inherent in international politics, and which could seriously hamper the operation of the Foundation in other regions.

A campaign for \$250,000 for the first year's operating expenses is now being sought by the Foundation, whose headquarters are located at 270 Madison Avenue, New York 16, N. Y. Founded seven years ago, the Foundation has made grants to various medical schools for under-graduate studies in tropical medicine and sponsored a field investigation of African sleeping sickness in Liberia. It is working toward the establishment of a full scale graduate school for tropical medicine in this country, open to all students to provide fully trained physicians for the Army, Navy and foreign economic development.

The research program of the Liberian Institute will be guided by a Scientific Committee made up of representatives of 17 universities, the Army and Navy medical services, the U. S. Public Health Service and industries directly interested in tropical medicine. This group includes:

1. Bowman Gray School of Medicine, Winston-Salem, N. C., Dr. Thomas T. Mackie.
2. Columbia University School of Public Health, New York, N. Y., Dr. Harold W. Brown.
3. Duke University School of Medicine, Durham, N. C., Dr. Donald S. Martin.
4. Harvard University Medical School, Boston, Mass., Dr. George C. Shattuck.
5. Harvard University School of Public Health, Boston, Mass., Brig. Gen'l. James S. Simmons.
6. Howard University College of Medicine, Washington, D. C., Dr. Joseph L. Johnson.
7. Johns Hopkins University School of Medicine, Baltimore, Md., Dr. Perrin H. Long.
8. Long Island College of Medicine, Brooklyn, N. Y., Dr. Jean A. Curran.
9. Meharry Medical College, Nashville, Tenn., Dr. Murray C. Brown.
10. New York University College of Medicine, New York, N. Y., Dr. Henry E. Meleney.
11. University of California Medical School, San Francisco, Cal., Dr. Hamilton H. Anderson.
12. University of Chicago School of Medicine, Chicago, Ill., Dr. William H. Taliaferro.
13. University of Michigan Medical School, Ann Arbor, Michigan, Dr. Malcolm H. Soule.

14. University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pa., Brig. Gen'l. Raymond A. Kelser.
15. University of Southern California School of Medicine, Los Angeles, Cal., Dr. James A. DeLamater.
16. Stanford University School of Medicine, San Francisco, Cal., Dr. Rodney Beard.
17. Tulane University of Louisiana School of Medicine, New Orleans, La., Dr. E. Carroll Faust.

In addition, the following organizations and services are represented on the Scientific Advisory Committee:

Dr. Sterling Brackett—American Cyanamid Co.
Brig. General George R. Callender—U. S. Army
Dr. Herbert Clark—Gorgas Memorial Institute
Dr. Justus B. Rice—Winthrop Chemical Co.
Dr. Carroll E. Roach—Eli Lilly Co.
Dr. Edward I. Salisbury—United Fruit Co.
Capt. Joseph Sapero—National Naval Medical Center
Dr. James A. Shannon—Squibb Institute for Medical Research
Dr. Willard H. Wright—National Institute of Health

THE FOURTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA

The decision that the Fourth International Congress on Tropical Medicine and the Fourth International Congress on Malaria should meet jointly in the United States of America in the spring of 1948 was reached after consultation with officers of the two organizations. The advantages in a combined congress of these international bodies were demonstrated when they met together in Amsterdam in 1938. The Fourth International Congresses on Tropical Medicine and Malaria, as the joint meeting has been designated, will meet in Washington from May 10 to 18, 1948. The Congresses will be under the sponsorship of the Department of State of the United States Government, which is issuing invitations to other Governments to send representatives. The Department of State as sponsor will have the cooperation of the following agencies and scientific societies interested in tropical medicine:

- Bureau of Medicine and Surgery, U. S. Navy
- Department of Agriculture
- National Research Council
- Office of the Surgeon General, U. S. Army
- United States Public Health Service
- Veterans Administration
- American Academy of Tropical Medicine
- American Association for the Advancement of Science
- American Association of Economic Entomologists
- American College of Physicians
- American Dermatological Association
- American Medical Association
- American Public Health Association
- American Society of Parasitologists
- American Society of Tropical Medicine
- American Veterinary Medical Association
- Entomological Society of America
- Medical Society of the District of Columbia
- National Malaria Society
- Southern Medical Association

The arrangements are being made by an Organizing Committee composed chiefly of the representatives of the agencies and societies cooperating in sponsoring the Congresses. The officers of the Organizing Committee are:

- Chairman: Dr. Thomas Parran, Surgeon General, United States Public Health Service
- Vice-Chairman: Dr. George K. Strode, Director International Health Division, Rockefeller Foundation
- Vice-Chairman: Mr. Clarke L. Willard, Assistant Chief, Division of International Conferences, Department of State

Program Director: Dr. Rolla E. Dyer, Director, National Institute of Health

Executive Secretary: Dr. Wilbur A. Sawyer

Secretary: Mr. William L. Breese

Address of the Secretaries: Division of International Conferences, Department of State, Washington 25, D. C., U.S.A.

The President, Vice-Presidents, Honorary Presidents, and Honorary Vice-Presidents of the Congresses will be elected in the first plenary meeting, and the sections will likewise elect Chairmen and Vice-Chairmen in their opening sessions.

PROGRAM

There will be an opening plenary session at 11:00 A.M. on May 10 (Monday). The full program of scientific and plenary sessions, receptions, entertainment, and excursions will be available at time of registration. The scientific meetings will be organized in twelve sections listed below with their conveners. The program of each section is being arranged by the Program Committee and the several conveners, and invitations to present scientific papers are being sent out by the Organizing Committee. The conveners will open the first sessions of their sections.

SECTIONS AND CONVENER

- I Research and Teaching Institutions
Dr. Wilbur A. Sawyer, Convener
- II Tropical Climatology and Physiology
Dr. David B. Dill, Convener
- III Bacterial and Spirochetal Diseases
Dr. Thomas B. Turner, Convener
- IV Virus and Rickettsial Diseases
Dr. John R. Paul, Convener
- V Malaria
Dr. Mark F. Boyd, Convener
- VI Helminthic Diseases
Dr. W. W. Cort, Convener
- VII Protozoan Diseases
Dr. Ernest Carroll Faust, Convener
- VIII Nutritional Diseases of the Tropics
Dr. Thomas T. Mackie, Convener
- IX Tropical Dermatology and Mycology
Dr. Fred D. Weidman, Convener
- X Tropical Veterinary Medicine
Dr. R. A. Kelser, Convener
- XI Public Health
Dr. Henry E. Meleney, Convener
- XIII Medical and Veterinary Entomology
Dr. Fred C. Bishopp, Convener

MEMBERSHIP AND REGISTRATION

There will be the following classes of participants:

Official Delegates: Official representatives of governments.

Institutional Delegates: Representatives of invited universities, societies, and scientific and philanthropic organizations interested in tropical medicine.

Members: Physicians, scientists, and other professional persons qualified in tropical medicine.

The above three classes of members comprise the professional group. With the exception of the Official Delegates of Governments they will be subject to a registration fee of ten dollars. All members in these professional classifications will have the full rights and privileges of the Congresses and will afterward receive the Report of Proceedings.

Associates: Students, non-professional persons interested in tropical medicine, and members of the families of professional members. Associates will have the privilege of attending general and sectional meetings, but will not have the right to participate in discussions or to vote. The registration fee for Associates is five dollars, unless they give notice at the time of registration that they wish to receive the Report of Proceedings, in which case the fee will be ten dollars.

Sustaining Members: Persons, firms, corporations, or organizations that have assisted in financing the Congresses by making the contribution specified by the Organizing Committee for this class of membership.

Registration will commence at 11:00 A.M. on Sunday, May 9, the day preceding the opening of the Congresses, and will be resumed at 9:00 A.M. on Monday, May 10. The exact places of registration and of Official Headquarters will be announced later.

LANGUAGES

The official languages of the Congresses are English, French, and Spanish. Any member finding it necessary to speak in a non-official language will be expected to provide an interpreter. All papers must be in one of the official languages.

INQUIRIES

Inquiries regarding the Congresses should be sent to the Executive Secretary of the Organizing Committee, Fourth International Congresses on Tropical Medicine and Malaria, Division of International Conferences, Department of State, Washington 25, D. C.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgement must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

- PABLO COVA-GARCÍA. *Notas sobre los Anofelinos de Venezuela y su Identificación.* pp. 208. 42 numbered and 25 unnumbered full page figures. XII Conferencia Sanitaria Panamericana. Cuadernos amarillos No. 1. Publicaciones de la Comisión Organizadora. Editorial Grafalit, Caracas, Venezuela, 1946.
- ACTA TROPICA. Review of Tropical Science and Tropical Medicine issued in collaboration with eminent Swiss and foreign specialists by R. Geigy, A. Gigon, F. Speiser, R. Tschudi, Professors in the University of Basel. Vol. 2, 1945. pp. 1-384. Price Swiss francs 30.00. Verlag für Recht und Gesellschaft, A. G., Basel.
- HERMAN MOOSER. *Die Beziehungen des murinen Fleckfiebers zum Klassischen Fleckfieber.* Supplementum 4. Acta Tropica pp. 1-87. Verlag für Recht und Gesellschaft, A. G. Basel.
- CLARENCE ANDERSON HUBBARD. *Fleas of Western North America. Their Relation to the Public Health.* pp. i-x; 1-533. Plates and illustrations. Price \$6.00. Iowa State College Press, Ames, Iowa.
- PROF. DR. P. C. FLÜ. *The Bacteriophage. A historical critical survey of 25 years research.* Vol. XVII. Acta Leidensia Editio Cura et Sumptibus Scholae Medicinae Tropical. Universitaire pers Leiden, MCMXLVI.

BOOK REVIEW

Fleas of Western North America, Their Relation to the Public Health. Clarence Anderson Hubbard, formerly Head of The Department of Biology, and Director of the Pre-Medic Curriculum of Pacific University, Forest Grove, Oregon. Pp. IX + 533. Iowa State College Press, Ames, Iowa. 1947. Illustrated. (\$6.00).

This comprehensive volume is divided into three parts, the first of which contains brief chapters on the students of Western American fleas, the medical importance of fleas, field and laboratory technique, and the anatomy of the flea in relation to its taxonomy.

Part II, Systematic Classification, occupies more than half of the volume, and in it are listed 246 species and sub-species of fleas occurring in the area west of the 100th meridian. These are divided among 66 genera and five families. The comparative richness of the flea fauna of this area is indicated by the fact that only 33 genera and some 55 species are known from east of the 100th meridian. For each species are given a short taxonomic description, the known range, full host records, and some notes on biology, abundance, and medical importance. Original drawings of the important anatomical characters for nearly all species are a valuable feature. These are conveniently inserted with the descriptive matter. At the end of this section are two useful tables showing the geographical distribution of fleas, one for the western states, the other for the eastern states.

Part III contains an extensive index to the host animals from which the different fleas have been recorded. This part also contains records of the species and location of animals in which plague infection has been found, as well as notes on their relation to tularemia and murine typhus.

The author observes that much of the systematic work on fleas is unsatisfactory and this seems to be reflected in the multiplicity of genera. It may be noted that about two-thirds of the recognized genera contain only one or at most two species. To one who is not a specialist in this order of insects this appears confusing and unnecessarily difficult. The only methods of flea control mentioned are flea powders for adults and salt water or kerosene for control of larvae. The volume is handsomely printed and is an important and valuable contribution to the subject.

W. V. KING

ORAL EMETINE IN THE TREATMENT OF INTESTINAL AMEBIASIS

· BLISS C. SHRAPNEL, M.D.

*From the Gorgas Memorial Laboratory, Republic of Panama, and
The Medical Service, Gorgas Hospital, Ancon, Canal Zone*

A previous paper (1) reported the trial use of emetine hydrochloride, orally, in the form of enteric-coated tablets, for the treatment of intestinal amebiasis. This study presents further observations on the use of this drug.

Emetine hydrochloride is known to be an effective amebicidal agent. Dobell and Laidlaw (2), St. John (3), and Bonnin and Aretas (4) have shown, by experimental methods, that emetine or its salts has a direct amebicidal action which is effective in very high dilutions; 1-1,000,000 to 1-5,000,000.

Table 1 summarizes the available information of the amebicidal activity, *in vitro*, of some of the drugs used in the treatment of amebiasis. It can be seen that emetine is a much more effective amebicidal agent than any of the newer drugs on which *in vitro* studies have been carried out.

Since emetine has a powerful, direct, amebicidal action, a property demonstrated in *in vitro* studies, oral therapy should eradicate the parasite. However, its concomitant powerful emetic action, resulting in severe salivation, nausea, and vomiting, has prevented administration of sufficient amounts to prove effective parasitologically. A few attempts have been made to provide protective coatings such as keratin or salol, or to combine the salt with other drugs in the form of emetine bismuth iodide, or emetine antimony iodide, in order to lessen the emetic effect. These results have, on the whole, not been successful.

Rogers, (8), in 1912, advocated the subcutaneous administration of the hydrochloride salt, thereby avoiding much of the nausea and vomiting which usually accompanied the oral administration. There was widespread acceptance of this method of therapy, and, in general, the results have justified the continued use of the drug. It has been shown by clinical experience, and animal experimentation, that it is a toxic drug with a cumulative action when administered in this manner.

Because of the toxicity of subcutaneous emetine, its therapeutic range has been limited, and according to Craig (9), the amount which can be safely given is, in the majority of cases, insufficient to permanently eradicate the parasite. Current opinion has limited its use to the control of symptoms, or as an adjuvant to the iodine and arsenic compounds for eradication of the parasite. Emetine subcutaneously is still the drug of choice in the management of extra-intestinal amebiasis.

The drug used in this study was emetine hydrochloride, prepared by Eli Lilly Company, and consisted of "enseals", or enteric-sealed tablets, to be administered orally. Each tablet contained one-third grain of alkaloid, and was designed to release its contents into the lower bowel approximately three to four hours after ingestion, thus avoiding the emetic effect of its presence in the stom-

ach, and upper intestinal tract. Little is known of the amount of absorption of emetine that can occur from the large bowel, but from the results of this study it is believed to be minimal.

TABLE 1
Amebicidal Activity, In Vitro, of Drugs Used in Treatment of Amebiasis

DRUG	AMEBICIDAL CONCENTRATION 48 HOURS	INVESTIGATOR
Chiniofon	1-500	Kessel (1928) (5)
Carbarsone	1-4,000	Leake (1932) (6)
Vioform	1-10,000	Leake (1932) (6)
Emetine hydrochloride	1-1,000,000	Dobell, Laidlaw and Bishop (1928) (7)
Emetine hydrochloride	1-1,000,000	St. John (1932) (3)

TABLE 2

	*DYSENTERY ACUTE	DYSENTERY CHRONIC	"CARRIER" STATE	TOTAL
Children.....	2	2	1	5
Adults.....	6	13	6	25
Total.....	8	15	7	30

PROCEDURES AND CLINICAL MATERIAL

A total of thirty cases of intestinal amebiasis were treated with emetine hydrochloride in the form of enteric-sealed tablets, orally, in this series. Included in this group are Latin Americans, British West Indians, and North Americans, of both sexes, and of age groups ranging from 17 months to 66 years. There were acute and chronic dysenteries, as well as "carriers," as given in Table 2.

The patients were all hospital admissions although not all were primarily for amebiasis. Some cases were discovered on routine stool examinations and some by proctoscopic examinations. There was no attempt at selection of cases, except that none were used if treatment with emetine hydrochloride subcutaneously had already been begun, and none were used unless the presence of *E. histolytica* in the stool was verified by the author. All cases, except one, were given a complete course of treatment, as hospital patients, under the authors' direct supervision. The exception was case No. 27 (Table 4), a colored female adult, employed as laundress by the author, and treated as ambulatory, but under daily direct observation.

A standardized dosage schedule was used throughout, as follows:

Children: One tablet ($\frac{1}{2}$ grain) of emetine hydrochloride, orally, three times a day for 12 days. Total: 12 grains in 12 days.

Adults: Two tablets ($\frac{1}{2}$ grain) of emetine hydrochloride, orally, three times a day for 12 days. Total: 24 grains in 12 days.

* Terminology of War Department Tech. Bull. 759, May, 1945—*Amebic dysentery:* Cases of amebiasis with intestinal symptoms and abnormal stools which contain motile amoebae. *Carrier of E. histolytica:* Cases in which there are no symptoms and cysts alone are found.

As the avoidance of the emetic effect depends entirely on the protective coating of the tablets, particular care was taken that no cracked, chipped, or disintegrated tablets were administered. The tablets are small enough to be swallowed whole by small children.

It was pre-supposed that some tablets would be passed through the entire intestinal tract without liberating their contents, especially in cases of acute dysentery. This was found to be true in two cases in which tablets were seen in the rectal lumen during a proctoscopic examination. The standard dosage was maintained throughout, however, as in ordinary treatment it would be impractical to determine exactly how many tablets had dissolved, of the total given.

All patients were observed daily on ward rounds and questioned closely as to symptoms of abdominal cramps, diarrhea, nausea or vomiting, insomnia, joint pains, malaise. Insofar as practicable the following routine was established.

(1) Daily microscopic examinations of stools for amebae. Specimens of all stools passed by each case were examined daily by the author before, during, and for a few days following, the course of treatment. Most cases harbored other intestinal parasites, notably helminths, and were given vermifuges and saline purges before and following the emetine. These stool specimens were also carefully examined for amebae.

(2) Daily cultures of stools for micro-organisms.

(3) Daily urinalysis.

(4) Blood pressure readings.

(5) Complete blood count every third day.

(6) Electrocardiogram every three days, except on infants.

(7) Proctoscopic examinations before and after treatment.

(8) Daily count of number of stools passed.

All cases were allowed out of bed as much as desired. No restrictions as to diet were followed, the patients usually taking the full diet.

IMMEDIATE RESULTS IN CHILDREN

There were five cases of intestinal amebiasis in children, (Table 3) of ages ranging from $1\frac{5}{12}$ to 9 years. There were two cases of acute dysentery, two of chronic dysentery, and one "carrier" state. All harbored other intestinal parasites, notably helminths, and presented the picture of malnourishment, underdevelopment and secondary anemia. They were given the usual vermifuges and purges as well as oral emetine hydrochloride. Case No. 3 was admitted critically ill with acute amebic dysentery, a sahli hemoglobin of less than 10 percent, and a total red cell count of 1,020,000. She required two blood transfusions prior to therapy for the parasites.

None of these children manifested vomiting or toxic manifestations during the administration of oral emetine. All showed a steady clinical improvement. The amebae disappeared from the stools between the third and fifth days of treatment, appetites improved, and the frequency of bowel movements diminished. A moderate diarrhea persisted in case No. 2. He was given a course of sulfaguandine as pus cells were noted in the stool specimens. The diarrhea promptly subsided and the pus cells disappeared.

TABLE 3
CHILDREN
One grain daily for 12 days

CASE NO.	AGE	DIAGNOSIS, AND PARASITES FOUND	SYMPTOMS	Hb % SAHLI	IMMEDIATE RESULT	RE-EXAMINATION			
						Time since Rx	Stool Smears	Hb % Sahli	Comment
1	3	Amoebic dysentery, chronic. Anemia, secondary <i>E. histolytica</i> troph. <i>S. stercoralis</i> <i>T. trichiuris</i>	Occasional convulsive attacks associated with listlessness and apathy for past 3 months. Grinds teeth.	56	No amoebae after 3rd day. Appetite improved during hospital stay. No convulsions. No toxic symptoms.	4 mos.	Negative for amoebae	00	Has been perfectly well since treatment, except for an attack of impetigo.
2	1½	Amoebic dysentery, acute Anemia, secondary <i>E. histolytica</i> troph. <i>T. trichiuris</i> <i>G. lamblia</i>	Has had diarrhea for 3 months, but 4 days ago became worse, with blood and mucus. Pus noted in stools. Weighs 18 lbs.	38	Amoebae disappeared after 5th day of Rx, but pus and mucus still present. Given course of sulfaguanidine following emetino. No toxic symptoms.	8 mos.	Negative for amoebae <i>T. trichiuris</i> ova present	78	Father states child has been in excellent health since discharge. Appetite has been very good, child has gained weight. Has not had any diarrhea or fever. (Weight 25 lbs.)
3	5	Amoebic dysentery, chronic severe. Anemia, secondary, severe. <i>E. histolytica</i> , troph. <i>E. coli</i> , troph. <i>N. americanus</i> <i>S. stercoralis</i> <i>T. trichiuris</i> <i>G. lamblia</i>	Child has been passing worms in stool for 7 months. Also has vomited "long worms." Only treatment has been "muy grande" purges 3 or 4 times in past few months. Child has gradually become weak and pale.	10	No amoebae after 4th day of Rx. Critically ill on admission. Required 2 transfusions prior to therapy for parasites. No toxic symptoms manifested during emetine treatment. Early morning stools contained large numbers of <i>T. trichiuris</i> worms after 5th day of emetine.	4 mos.	Negative for <i>E. histolytica</i> Positive for: <i>N. americanus</i> <i>T. trichiuris</i> <i>S. stercoralis</i> <i>E. coli</i> cysts	58	Has had no diarrhea, fever, or abdominal pain, but has had "catarro." Has gained weight. Anemia is good.

4	0	"Carrier" of <i>E. histolytica</i> Vaginitis, gonorrheal Anemia, secondary <i>E. histolytica</i> cysts <i>N. americanus</i> <i>S. stercoridis</i> <i>T. trichiuris</i> <i>E. coli</i> cysts <i>G. lamblia</i>	Vaginal discharge for past month. No diarrhea, but has occasional abdominal cramps.	72	No cysts found after 3 days Cultures for amebiasis negative after 3rd day. Averaged 1 stool daily, but on 6th day had 4 stools. No toxic symptoms.	6 mos.	Negative for <i>E. histolytica</i> Positive for: <i>S. stercoridis</i> <i>E. coli</i> , cysts	80	No symptoms. Has been perfectly well at present.
5	4	Amebic dysentery, acute Anemia, secondary <i>E. histolytica</i> , troph. <i>E. coli</i> , troph. <i>T. trichiuris</i> <i>A. lumbricoides</i> <i>Trichomonas</i> sp.	About a month ago began to have bloody stools associated with a frequent diarrhea.	59	No <i>E. histolytica</i> trophozoites found in stools after 3rd day of Rx. Several large numbers of <i>T. trichiuris</i> worms in early morning stools after 3th day of Rx. Frequency of stools diminished gradually. Gained 3 lbs. in weight during treatment. No toxic symptoms.	4 mos.	Negative for amebiasis. Positive for: <i>A. lumbricoides</i> <i>T. trichiuris</i> (few)	59	Stool discharge still has been well. Trophozoites, no vomiting, diarrhea, or abdominal pain. Gave well, returned to own weight, and is active.

Case No. 4, a nine year old colored girl, was admitted for gonorrhoeal vaginitis and was found to harbor a total of seven intestinal parasites, including *E. histolytica* cysts. She denied having had diarrhea, but did have occasional abdominal cramps. This was the only "carrier" state in children. She was treated with intramuscular penicillin, and oral emetine, concomitantly, without manifesting toxic symptoms. *E. histolytica* cysts or trophozoites could not be found in the stools, by smear or culture methods, after the fifth day of emetine treatment.

A noteworthy side effect in children treated with oral emetine, was the expulsion of large numbers of adult *Trichiuris trichiuris* in the early morning stools of cases No. 3, and No. 5, which were heavily parasitized with this worm. Both of these cases had persistent rectal prolapse occurring with each bowel movement. Large numbers of adult *Trichiuris trichiuris* could be seen on the rectal mucosa when it prolapsed. Consequent to the fifth day of oral emetine treatment, the early morning stools of these 2 children contained large numbers of these worms. As treatment continued, the frequency of the stools diminished, and there were noticeably fewer worms to be seen on the prolapsed rectal mucosa. The children gained weight gradually, and the rectal prolapse finally ceased. *Trichiuris trichiuris* ova were still present, however, in stool specimens examined several days following the course of oral emetine.

IMMEDIATE RESULTS IN ADULTS

Twenty-five adults, of ages from 18 to 66 years, were treated with a standardized dosage of two tablets of emetine hydrochloride, orally, three times a day for 12 days, a total of 24 grains in 12 days. (Table 4).

This series included 6 cases of acute dysentery, 13 of chronic dysentery, and 6 of the "carrier" state.

There were no serious toxic symptoms manifested in this group. No vomiting occurred in any case, care being taken to administer only complete, unbroken tablets. The diarrhea of the acute cases subsided rapidly, the number of stools reducing to 2 or 3 daily, then to none, or one, daily, by the end of the course of treatment. The abdominal cramps and tenesmus responded quickly, usually by the second or third day. Some of the chronic and "carrier" cases, who had not had diarrhea, began to have two or three soft stools daily during the treatment, but this was not accompanied by cramps or tenesmus. This was not considered a toxic effect. The trophozoites of *E. histolytica* could not be found in stool examinations, on the average, by the third day of treatment, but cyst forms could be found as late as the sixth day.

The patients having amebic ulcerations of the rectum were examined periodically by proctoscope and steady healing was evident. All cases, except two, showed complete healing at the end of treatment. One of these (No. 15), with large rectal ulcerations before treatment, showed a slight granularity in the appearance of the rectal mucosa at the end of treatment, but direct smears were negative for amebae. A recheck proctoscopic examination, one month later, showed a normal rectal mucosa throughout. The rectal lesions present in the other case (No. 25), were incompletely healed at the end of the oral emetine treat-

ment, but direct smears were negative for amebae. He was given a course of sulfadiazine for 10 days, and healing was complete.

There were no evidences of toxic effects of emetine on the myocardium. The blood pressure readings, pulse rates, and cardiac sounds showed no significant changes. No effects were noted on the electrocardiograms taken before, during, and after treatment. Case No. 26 had an electrocardiographic record of auricular fibrillation one year previous to emetine treatment. His electrocardiogram, taken prior to treatment, was essentially normal, and there was no demonstrable effect noted on repeated electrocardiograms taken during and following the administration of 24 grains of emetine, orally, in 12 days.

There were no significant changes in the urine during treatment. No evidence of albuminuria, glycosuria, acetoneuria, gross changes in specific gravity, or cellular constituents occurred.

All patients were able to take the regular diet while under treatment without nausea or vomiting, and were entirely ambulatory. The one case (No. 27) allowed to continue her work as a laundress while taking 2 grains of emetine orally each day, felt well during the entire course of treatment. Her abdominal cramps, tenesmus, and diarrhea subsided by the second day of treatment and there was a definite increase in her appetite.

There were no complaints of joint pains, nervousness, insomnia, or neuritides. The absence of joint pains was particularly noted as it is often encountered when emetine is administered subcutaneously.

Other drugs, including oral atebine, hexylresorcinol crystalloids, sulfaguanidine, sulfadiazine, have been given, along with the oral emetine without interference or toxicity.

Most cases manifested secondary anemias to varying degrees. As there were often other intestinal parasites present, the amount of anemia caused by the amebae could not be accurately determined. All received treatment for the other parasites as well as the amebae and all showed improvement in the blood picture.

Aside from the mild irritative effect of the emetine in the intestine, producing two or three soft stools daily, but without cramps or tenesmus, in some cases, no toxic effects were noted in this series.

RECHECK STUDIES

The criteria of permanent cure of intestinal amebiasis are not definite. The inability to demonstrate cysts or trophozoites in the stools, plus clinical improvement, after a variable period of time since treatment is completed, is usually interpreted as a cure. The frequency of follow-up rechecks, and the method of stool examinations, has varied with different investigators. The possibility of re-infestation of the patient when he returns to his original environment cannot be eliminated. The longer the interval between recheck studies, the more possible it is that re-infestation may occur.

Twenty-four of the thirty cases treated with oral emetine were re-admitted as hospital patients for recheck studies, from 2 to 9 months after receiving the drug.

TABLE 4
ADULTS
Two grains daily for 12 days

CASE NO.	AGE	DIAGNOSIS, AND PARASITES FOUND	SYMPTOMS	PROCTOSCOPIC	Hb % SAHLI	IMMEDIATE RESULT	RE-EXAMINATION				
							Time since Rx	Stool Smears	Proctoscopic	Hb % SAHLI	Comment
23	33	Amebic dysentery, chronic Hernia, right inguinal Sinusitis, chronic <i>E. histolytica</i> , troph. <i>S. stercoralis</i>	Was treated 4 months ago for amebic dysentery with Carbarsone. Since then has had diarrhea off and on, and pains in right inguinal region. Has 5 to 6 stools daily now.	Normal mucosa	80	No amebae found after 3rd day. Stools subsided to 2 or 3 daily. Had right inguinal herniorrhaphy after receiving emetino Rx. Stools checked periodically during 6 weeks hospitalization. All were negative for amebae.	8 months	Negative for amebae	Normal mucosa	80	Has had occasional pains in right inguinal region, at site of operation, but otherwise has been well. No abdominal cramps, or diarrhea.
29	33	Amebic dysentery, chronic Anemia, secondary <i>E. histolytica</i> , cysts & troph.	5 months ago was treated for amebic dysentery with carbarsone. At that time he had "ulcers in the rectum." For past 2 weeks he has had diarrhea and abdominal pains. Thinks he might have "amebas" again.	Several areas of small ulcerations with one long, shallow ulcer. After Rx: Normal mucosa.	73	No amebae found after 4th day of Rx. No toxic symptoms.	3 months	Negative for amebae	None	80	Has felt well. No further diarrhea. Has gained weight.

30	41	Amoebic dysentery, chronic <i>E. histolytica</i> , cyst & troph.	Admitted only for recheck on previously treated amebiasis with emetine. <i>History:</i> Had acute amoebic dysentery, with rectal lesions in 1944. Treated with oral emetine, 2 grains daily for 6 days. This repeated 6 days later. Rectal lesions healed. Symptoms disappeared. Rechecked in 1 month, cysts found. Denied symptoms. No rectal lesions. Given 2 grains daily for 8 days. Rechecked 5 months later. No symptoms. No rectal lesions. Gained 15 lbs. in weight. Trophozoites found after emetine purge. Treated this time with 2 grains daily for 12 days.	85	No symptoms. No signs of toxicity.	6 months	Negative for amebae	Normal mucosa	89	Long history of amebiasis. Treated twice with oral emetine with dosage of less than 10 days, resulting in symptomatic cure, but parasite not eliminated. Last dosage of 2 grains daily for 12 days sufficient to eradicate parasite from stools up to 5 months following treatment.
23	32	Acute dysentery, Syphilis, L-IV <i>E. histolytica</i> , troph.	For past 2 months has had constant diarrhea of watery, dark brown stools, sometimes with streaks of blood. Occasional sharp, shooting pains in abdomen.	81	Diarrhea subsided on 5th day of Rx. No amebae found after 4th day. No toxic symptoms. X-ray of liver area normal. Heart enlarged in transverse diameter.	3 months	Negative for amebae	None	80	No symptoms. "Sleeps good, and eats good." Has gained 8 lbs. X-ray of liver and diaphragm normal. Heart enlarged in transverse diameter.
21	45	Amoebic dysentery, chronic <i>E. histolytica</i> troph.	Onset 6 weeks ago, in Guatemala, of severe diarrhea. Received treatment of Carbarsone, and Vioform, of one week each, without effect. Since then has had repeated bouts of diarrhea at about weekly intervals.	93	No toxic symptoms. Continued to have 3 to 4 stools daily throughout course of emetine. No abdominal symptoms. Appetite good.	4 months	Negative for amebae	Normal mucosa	90	Has had no symptoms since discharge. Has gained 20 lbs. in weight, and states he has a definite increased sense of well-being. X-ray of diaphragm normal.

TABLE 4—Continued

CASE NO.	AGE	DIAGNOSIS, AND PARASITES FOUND	SYMPTOMS	PROCTOSCOPIC	Hb % SAHLI	IMMEDIATE RESULT	RE-EXAMINATION				
							Time since Rx	Stool smears	Proctoscopic	Hb % Sahli	Comment
25	36	"Carrier" of <i>E. histolytica</i> <i>E. histolytica</i> , cysts	Admitted to hospital for operation on nose. Had history of amebiasis 3 years ago, and requested recheck of stools.	Few ragged ulcerations present.	93	Had 3 stools daily while on ometino Rx. Proctitis did not heal completely at end of ometime course. Given course of sulfadiazine, and healing was prompt. No toxic symptoms.	Not obtained				Seen outside hospital and stated he felt entirely well, but was unable to enter hospital for recheck studies.
26	42	"Carrier" of <i>E. histolytica</i> Myositis, chelonio, cervical post-traumatic. <i>E. histolytica</i> , cysts.	For past 6 months has been extremely tired at end of working day. No history of abdominal pains or diarrhea.	Normal mucosa	88	No toxic symptoms. No increase in bowel movements.	3 months	Negative for amebae	Normal mucosa	92	Has felt generally better, but not entirely well. Has not had any abdominal symptoms, or diarrhea.
27	26	Amebio dysentery, acute Anemia, secondary <i>E. histolytica</i> , troph.	Has had severe diarrhea, abdominal pains, and vomiting for past 2 weeks. Never had this before.	Normal mucosa	69	Entire course of ometino Rx. given as outpatient while employed as laundress by author. Diarrhea subsided in 2 days of treatment. No further vomiting or abdominal cramps. Appetite improved.	2 months	Negative for amebae	None	80	This pt. employed as laundress by the author. Observed for 3 month period after ometino Rx., and had no recurrences of abdominal symptoms or diarrhea. Appetite good, and gain in weight and general health was noticeable.
17	25	Amebio dysentery, chronic Anemia, secondary <i>E. histolytica</i> troph. <i>N. americanus</i> <i>Trichomonas</i> sp.	Had "dysentery" lasting 6 months, about 2 years ago. For past 6 days has had 3 to 5 bloody stools daily.	Many varied ulcerations present. Direct smear positive for <i>E. histolytica</i>	50	Diarrhea subsided to 1 or 2 stools daily, by 3rd day of Rx. No amebae seen after 5th day. No toxic symptoms.	Not obtained				

18	30	Amebic dysentery, acute Anemia, secondary Syphilis, L-IV <i>E. histolytica</i> troph. <i>G. lamblia</i>	2 weeks ago developed epigastric pain, anorexia, malaise, associated with 4 to 5 watery stools daily. This has increased to 10 to 12 daily, with 4 to 5 at night. Stools are stained with blood.	Several large irregular ulcerations. 3 days after Rx. Normal mucosa to 20 cm.	75	Diarrhea reduced to 3 stools daily by 4th day of Rx. No mme-bae found after 4th day. No toxic symptoms.	9 months	Negative for amebae	Normal mucosa	82	States he has felt very well since treatment. No symptoms of diarrhea whatever. Has gained weight.
19	41	Amebic dysentery, chronic Anemia, secondary <i>E. histolytica</i> , cyst & troph. <i>G. lamblia</i> <i>D. fragilis</i> <i>T. trichiuris</i> <i>C. meynlii</i>	Has had pains in R.U.Q. of abdomen, and flank, for past month, associated with gas pains. No diarrhea.	Granular appearing mucosa with several pinpoint easily bleeding areas.	72	Atrbrine given for giardiasis, simultaneously with emetine, for first 5 days. No toxic symptoms developed. No amebae found after 6th day.	Not obtained				
20	24	Amebic dysentery, chronic <i>E. histolytica</i> , cysts & troph. <i>E. coli</i> , cysts	At some Cakhova and developed sudden pains in R.U.Q. of abdomen, with vomiting.	Normal mucosa	90	No amebae found after 2nd day of Rx. No toxic symptoms.	3 months	Positive for: <i>E. coli</i> cysts Negative for <i>E. histolytica</i>	Normal mucosa	90	Has gained 8 lbs. in weight. Feels well. No diarrhea.
21	23	Amebic dysentery, acute Anemia, secondary <i>E. histolytica</i> , troph.	Onset 3 days ago of colicky abdominal pains with persistent diarrhea. No blood noted in stools.	Normal mucosa	70	Had 3 or 3 stools daily up to 10th day of Rx., then 0 or 1 daily. No mmebae found after 2nd day. No toxic symptoms.	Not obtained				
22	19	"Carrier" of <i>E. histolytica</i> <i>E. histolytica</i> , cysts <i>E. coli</i> , cysts <i>N. americanus</i> <i>S. stercoralis</i> <i>T. trichiuris</i>	Denies any, and all symptoms. Parasites found on routine pre-employment food-handler examination.	Normal mucosa	86	No cysts found after 6th day of Rx. No toxic symptoms.	7 months	Negative for amebae. Positive for: <i>N. omer-ticinus</i>	None	90	No symptoms. Gained weight. Blood picture improved.

TABLE 4—Continued

CASE NO.	AGE	DIAGNOSIS, AND PARASITES FOUND	SYMPTOMS	PROCTOSCOPIC	Hb % SAHLI	IMMEDIATE RESULTS	RE-EXAMINATION					
							Time since Rx	Stool smears	Procto-scopic	Hb % Sahli	Comment	
13	43	Amoebic dysentery, chronic <i>E. histolytica</i> , cysts & troph. <i>N. americanus</i> <i>S. stercoralis</i> <i>E. coli</i> , cysts <i>D. fragilis</i> , troph.	Has had "stomach trouble" for 4 years. Has epigastric pain associated with belching. No vomiting or diarrhea, but has lost weight.	Normal mucosa	86	No amoebae found after 7th day of Rx. No toxic symptoms. No change in "stomach trouble."	Not obtained					
14	60	"Carrier" of <i>E. histolytica</i> Malaria, clinical Anemia, secondary <i>E. histolytica</i> , cysts <i>E. coli</i> , cysts	Fever, chills, and generalized aches and pains, for past 2 days. Vomited. Severe headache. <i>E. histolytica</i> cysts found on routine stool examination.	Several pinpoint easily bleeding areas scattered in rectum. 12th day of Rx: Normal appearing mucosa	74	No amoeba found after 3rd day. Fever responded to atabrine. No toxic symptoms.	4 months.	Negative for amoebae	Normal mucosa	78	Has had no symptoms since treatment. Feels good. Has gained 16 lbs. in weight.	
15	54	Amoebic dysentery, acute Diabetes mellitus <i>E. histolytica</i> troph. Anemia, secondary	Known diabetic. Recently had all teeth extracted and has been unable to eat properly, and has been having 3 to 4 watery stools daily. Has lost 10 lbs. in weight, and feels weak.	<i>Before Rx:</i> Large rectal ulcerations. 12th day of Rx: Mucosa slightly granular in appearance. No ulcerations. Direct smear negative.	60	No amoebae seen in stools after 3rd day of Rx. No toxic symptoms. Recheck proctoscopic one month after discharge showed normal mucosa.	4 months	Negative for amoebae	Normal mucosa	80	No further diarrhea since treatment. Has gained weight, and feels good.	

16	19	Amebic dysentery, chronic Malaria, tertian Syphilis, L-IV Anemia, secondary <i>E. histolytica</i> , cysts & troph. <i>E. coli</i> , cysts & troph. <i>E. nana</i> , troph. <i>N. americanus</i> <i>S. stercoralis</i> <i>T. trichiuris</i>	Has had chills, fever, headache and pains in back for past 5 days. No diarrhea or abdominal pains.	Normal mucosa	64	No amebae found after 2nd day of Rx. No toxic symptoms.	9 months	Negative for amebae. Positive for: <i>N. omer-icanus</i> <i>S. ster-coralis</i> <i>T. tri-chiuris</i>	Nong	70	Has had no symptoms since treatment. Has felt well. Appetite good.
10	22	Amebic dysentery, chronic Cellulitis, acute, supp. left leg. Tonsillitis, acute Anemia, secondary <i>E. histolytica</i> troph.	Admitted for treatment of cellulitis of leg. <i>E. histolytica</i> trophozoites found on routine stool examination. Denies abdominal symptoms or diarrhea.	Normal mucosa	68	No amebae found after 3rd day of Rx. No toxic symptoms.	5 months	Negative for amebae	Normal mucosa	80	No symptoms. Feels entirely well. Anemia improved.
11	55	"Carrier" of <i>E. histolytica</i> Hypertensive cardiovascular disease, with right hemiplegia. <i>E. histolytica</i> cysts <i>E. coli</i> cysts <i>G. lamblia</i> <i>Trichomonas</i> sp.	Had a "stroke" 5 days ago, and is unable to walk. States she has been constipated for years, and never has had abdominal pains or diarrhea. Blood pressure: 210/100.	Not done	75	No cysts found after 5th day of Rx. No toxic symptoms. No change in blood pressure.	6 months	Negative for <i>E. histolytica</i> Positive for: <i>E. coli</i> , cysts <i>Trichomonas</i> sp.	Mucosa mildly hypertrophic. Suggestive of "strawberry" color.	80	No abdominal symptoms. Is still troubled with constipation. Blood pressure 180/100. Is able to "get around a little."

TABLE 4—*Concluded*

CASE NO.	AGE	DIAGNOSIS, AND PARASITES FOUND	SYMPTOMS	PROCTOSCOPIC	Hb % SAHLI	IMMEDIATE RESULTS	RE-EXAMINATION				
							Time since Rx	Stool smears	Proctoscopic	Hb % Sahli	Comment
12	33	Amoebic dysentery, chronic Blepharo-conjunctivitis, bilateral <i>E. histolytica</i> troph. <i>E. coli</i> , cysts & troph.	Has had lacrimation and photophobia for 4 months. Has had abdominal pains and attacks of diarrhea for past 8 months. Has 5 bowel movements daily, with tenesmus and cramps every time he eats anything. Has lost 20 lbs. in weight.	<i>Before Rx:</i> Entire rectum covered with ulcerations, as large as 1 inch in length. <i>8th day of Rx:</i> Many ulcers present but appear to be healing. Mucosa bleeds easily. <i>12th day of Rx:</i> No ulcers seen. Few slightly erythematous areas present. Direct smears negative for amoebae.	84	No amoebae found after 4th day of Rx. Tenesmus subsided by 5th day of Rx. Pt. stated he felt steady improvement. No symptoms at all after 10th day of Rx. No toxic symptoms.	Not obtained				
6	44	Amoebic dysentery, acute Anemia, secondary, severe <i>E. histolytica</i> , troph.	Has had periodic attacks of diarrhea, and abdominal cramps for past 3 years. Recently has become much worse, and is losing weight rapidly.	Many typical amoebic ulcerations throughout lower bowel.	30	Diarrhea had subsided by 6th day. No amoebae found after 6th day. Pt. stated he felt much better on second day of Rx, although diarrhea still present. Proctoscopic on 14th day showed no lesions. No toxic symptoms.	7 months	Negative for amoebae	Normal mucosa	70	Has felt perfectly well. No further diarrhea or abdominal cramps. Has gained weight.

7	19	Anobio dysentery, chronic Malaria, clinical Anemia, secondary, severe <i>E. histolytica</i> , cysts & troph. <i>E. coli</i> , cysts <i>D. fragilis</i> , troph. <i>N. americanus</i> <i>S. stercoralis</i>	Has had fever, malaise, headache, for past 3 days. For past 2 years has had pale and yellow skin. Dense abdominal pains and diarrhea. Spleen enlarged.	No ulcerations. Mucosa pale.	53	Fever responded to atabrine. No amebae found after 3rd day of emetine Rx. Definite improvement of anemia while in hospital. No toxic symptoms noted.	7 months	Negative for amebae	None	80	No symptoms since discharge. Has been in good health. Marked improvement in anemia.
8	27	Anobio dysentery, chronic Yaws Collulitis, suppurative, of abdominal wall Anemia, secondary <i>E. histolytica</i> , cysts & troph. <i>E. coli</i> cysts <i>N. americanus</i> <i>S. stercoralis</i> <i>G. lamblia</i>	Has had fever, chills, and joint pains for 5 days. No nausea, vomiting, or diarrhea. Has been constipated.	Normal mucosa	73	No amebae found after 3rd day of Rx. Fever subsided with cellulitis. No toxic symptoms.	6 months	Negative for amebae	None	80	No symptoms since discharge. Has stool daily. Has gained 5 lbs. in weight and feels stronger. Blood picture improved.
9	31	Anobio dysentery, chronic Anemia, secondary <i>E. histolytica</i> , cysts & troph. <i>E. coli</i> , cysts & troph.	Has lost 10 lbs. in last 6 months. Feels run-down and chronically tired. No abdominal pains, but has periods of diarrhea.	Normal mucosa	69	No amebae found after 5th day of Rx. No toxic symptoms.	7 months	Positive for <i>E. histolytica</i> trophozoites following saline purge.	Normal mucosa	75	Has had no symptoms. Feels well, and has gained 12 lbs. in weight.

Complete interval histories and physical examinations were done, as well as laboratory studies. Proctoscopic examinations were done on adults, but not on children. Stool specimens were examined microscopically, using direct smear methods, by the author. Three smears, in saline suspension, from normally passed stools were each examined completely. If these were negative, a saline purge was administered, and three smears from each of several specimens were carefully examined. Cyst forms encountered were stained with iodine for identification.

If cysts or trophozoites of *E. histolytica* were found, irrespective of the time elapsed since original treatment, and irrespective of the presence or absence of symptoms, the case was considered a failure of oral emetine to cure.

The five children were rechecked in from 4 to 8 months following the original treatment. (Table 3). None were found to harbor *E. histolytica* cysts or trophozoites, but other intestinal parasites were present. Not one case had had any symptoms of diarrhea or abdominal pains in the interim. There was marked clinical improvement in all cases, manifested by increased activity and appetite, gain in weight, and improved general appearance. The blood picture showed marked improvement of the secondary anemia. The child (Case No. 3), who had had a hemoglobin of less than 10 percent at the time of treatment, showed a hemoglobin of 58 percent and a total count of 3,500,000, when rechecked 4 months later.

Nineteen of the twenty-five adults were obtained for recheck studies in from two to nine months following treatment with oral emetine. (Table 4). There was only one failure in this series. Case No. 9 was found to harbor *E. histolytica* trophozoites on examination of a saline purged stool. This was seven months following the original treatment. The patient denied any and all symptoms in the interim, had gained 12 pounds in weight, and felt so well he could not be convinced that he required further treatment.

All adults reported that they had felt entirely well since their treatment with oral emetine, and denied any symptoms of abdominal cramps or diarrhea. It was particularly noted that none complained of having had joint pains, and general feelings of tiredness, as is often noted when emetine is administered subcutaneously. All cases manifested normal findings on proctoscopic examinations, and the electrocardiograms manifested no changes. There was varying, but definite, improvement in the blood picture in all cases that had previously shown a secondary anemia.

There were no evidences of delayed toxic effects in either adults or children.

DISCUSSION

The present series of cases is small, but some conclusions can be drawn. Oral emetine, in the form used in this study, is a drug which is easy to administer, does not produce toxic symptoms in the dosage as reported herein, and provides effective results.

That the drug reaches the amebae in the ulcerated areas of the intestine can be demonstrated by following with the proctoscope the progress of a patient with

rectal ulcerations who is receiving the drug. A rapid decrease in the size of the ulcers, with healing, is evident. The presence of secondary infection of the amebic ulcerations will, however, prevent complete healing, although the amebae will disappear from mucosal scrapings, and the stools. The two cases in this series, whose rectal lesions failed to heal completely with oral emetine treatment, responded rapidly to sulfaguanidine and sulfadiazine, indicating a superimposed secondary infection of the amebic lesions.

There was no demonstrable evidence, in these cases, of absorption of sufficient amounts of emetine into the general system, from the large intestine to produce toxic symptoms. There were no cases of vomiting in the entire series, particular care being taken not to administer chipped or broken tablets, which would allow premature release of their contents into the stomach and upper intestinal tract.

The low or absent toxic effect of oral emetine in the dosage as used in these cases, is aptly demonstrated by the absence of toxic symptoms in the five children treated with oral emetine. The subcutaneous administration of one grain of emetine hydrochloride daily for twelve days, as was given orally, to these five children would have been dangerous, if not lethal. The adult dosage, although comparatively not as large as given to children, was twice the maximum recommended for subcutaneous administration, and failed to produce toxic effects.

It is thus possible, with the drug as used in this study, to administer orally, and without producing toxic effects, at least double the amount of emetine hydrochloride as could be done safely by subcutaneous administration.

The ability to administer a larger amount of emetine hydrochloride provides a method of maintaining a higher concentration of the drug in the intestinal tract, than was formerly possible by the subcutaneous route.

The results of this study indicate that the dosage used closely approaches the amebicidal concentration necessary to eradicate the parasite from the intestinal tract.

It is regretted that facilities were not available to study the concentration of free emetine hydrochloride obtained in the patients' stools by the dosage used in this series. The devising of a suitable method for determining the concentration obtained in the stools would facilitate the maintenance of adequate concentration of emetine in the intestinal tract to eradicate the amebae. The concentration of 1 to 1,000,000 is amebicidal *in vitro*, and the maintenance of this concentration should not be difficult with the drug as used in this series.

No difficulty was found in administering some other drugs to patients receiving emetine orally. Atabrine, hexylresorcinol crystoids, magnesium sulfate solution, ferric ammonium citrate solution, sulfaquanidine, sulfadiazine, and penicillin have all been given to patients in this series, without interference or toxicity.

There were no cases of extra-intestinal amebiasis encountered in this series. None of the cases restudied manifested any symptoms suggestive of liver infestation. X-rays of the liver and diaphragm were taken in some of the adults, but failed to show any demonstrable evidences of liver infestation, and was abandoned. It should be emphasized here that the drug, as used in this series, is probably not suitable for the treatment of extra-intestinal amebiasis.

It is possible that emetine hydrochloride enteric-sealed tablets may be an effective therapeutic agent against *Trichiuris trichiuris*. Two children in this series, receiving emetine orally, and heavily parasitized with this worm, passed large numbers of adult worms in their early morning stools. Controlled studies in a series of cases infested with this worm, and treated with oral emetine, would be of great value.

SUMMARY

Twenty-five adults and five children with intestinal amebiasis, including acute and chronic forms, were treated with emetine hydrochloride enteric-sealed tablets (Lilly), orally. Adults were given two grains daily for twelve days, and the children one grain daily for twelve days. No nausea or vomiting, nor other toxic symptoms were manifested. Nineteen of the adults, and all of the five children, were re-studied, as hospital patients, in from two to nine months following original treatment. Clinical cure was obtained in all cases, but parasitological cure failed in one adult, who was found to harbor *E. histolytica* trophozoites, seven months after receiving the drug.

The results obtained in this study indicate that emetine hydrochloride, in enteric-sealed tablets, administered orally, provides a concentration of emetine in the intestinal tract which closely approaches the amebicidal concentration necessary to eradicate the parasite, without producing toxic side effects.

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ALCOHOLIC EXTRACT MEDIUM FOR THE DIAGNOSIS AND CULTIVATION OF *ENDAMOEBIA HISTOLYTICA*¹

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INTRODUCTION

Alcoholic extract medium (1) is a type of medium prepared with extracts derived from tissue or egg preserved in 95 per cent ethyl alcohol. For the past three years, we have used various modifications of this type of medium in the diagnosis of *Endamoeba histolytica* infection and for continued maintenance of isolated strains in culture.

It is the purpose of this report to describe the modification of the original formula (1), which we have found most practical and efficient for the cultivation of *E. histolytica* and to present some data obtained from use of the medium.

PREPARATION OF MEDIUM

Alcoholic extract. The basic ingredient of alcohol extract medium is extract derived from tissue or egg preserved in 95 per cent ethyl alcohol. The types of tissues we have used to date will be discussed later. The preparation procedure is uniform regardless of source. We have selected as our standard a 10 per cent stock solution made by adding 10 cc. of tissue to 90 cc. of ethyl alcohol. The extract is made up in large quantities by placing 900 cc. of alcohol in a bottle and adding tissue cut into pieces to bring the total volume to 1,000 cc.

No special procedure is needed in obtaining the tissue. If guinea pig liver is to be preserved, the guinea pig is killed and the abdominal hair wet down with alcohol. The body wall is incised and the liver lifted out after clipping vessels and other attachments. The liver is held over the bottle of alcohol, clipped into pieces and dropped in. It appears to make no difference whether the gall bladder with the bile is included or not.

The bottle of tissue is shaken at intervals and is ready for use in 48 hours. No further handling procedure is necessary. In some instances, we have pulverized the tissue after several days hardening. This is not necessary. Even if the tissue is left in three or four large pieces, a potent extract is obtained. For the first few days after preservation, the supernatant liquid is cloudy, but all of this material finally settles out as an insoluble coagulum, leaving a clear supernatant which is used for making culture medium. It is drawn off by pipette as needed until the supply is exhausted. In some instances, we have used bottles of tissue as a source of extract for over two years and noted little loss in the potency of the extract.

Alcohol-free extract. In an earlier study (1), it was found that the essential

¹ The author wishes to acknowledge with thanks the assistance of Margaret C. Meyer, Tech. 4 Henry M. C. Chang and Sergeant Robert F. Vierra.

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nutrients and growth supporting substances are present in the alcoholic solution. The insoluble material from tissue is not needed. In the original procedure (1) one cc. of the alcoholic extract is placed in a test tube, which is then held in a boiling water bath to drive off the alcohol, a matter of a few minutes. Ten cc. of Ringer's solution is then added to the alcohol-free extract and the medium is ready for inoculation. No serum is added.

The quantity of alcoholic extract per tube of medium was standardized at one cc. This provides each tube of medium with the extract from approximately one-tenth gram tissue. When the alcohol is driven from the extract, there remains a small amount of oily fluid and precipitated waxy solid. The oily fluid emulsifies or dissolves in Ringer's solution, but the waxy solid appears to be insoluble.

New Modification of Medium

Medium prepared as described above is effective for *E. histolytica* cultivation, but has one defect. The waxy extract residue at the bottom of the tube may be obtained in samples drawn for amoeba examination, and in the microscope preparation will appear as globules which interfere with examination.

A modification was, therefore, devised in which the alcohol-free extract is taken up in hot two per cent agar and made into slants. A fluid overlay is added to the slants to complete the medium. Quantity preparation in batches of ten tubes is carried out as follows:

1. Place 10 cc. of alcoholic extract in a sterile 250 cc. Erlenmeyer flask. An inverted cotton-packed beaker makes a practical cover. Place the flask in a boiling water bath to drive off all of the alcohol, a matter of about five minutes.

2. Add 20 cc. of hot, melted two per cent sterile agar to the flask. The agar is dissolved in 0.5 per cent saline buffered to the desired pH (see overlay solution below) and sterilized in the autoclave. We find it convenient to autoclave 100 cc. lots in Erlenmeyer flasks, which are kept in stock and melted on the water bath when needed. After adding the agar, agitate the flask to incorporate the tissue extract. Some insoluble waxy and oily material may remain adherent to the flask, but its loss has no apparent effect on the efficiency of the medium. While still hot, pipette two cc. quantities of the agar medium into sterile 15 mm. x 150 mm. test tubes. Cool the tubes in a nearly horizontal position to obtain a steep slant. Reference will be made later to tests involving autoclaving the tubed agar before slanting. Autoclaving is not necessary if aseptic precautions have been followed.

3. *Overlay solution.* Add eight cc. of sterile buffered 0.5 per cent saline to each tube. No serum is used. This overlay was selected to replace the Ringer's solution formerly employed (1). It is simpler to prepare and has a greater pH stability. The same solution is used for the preparation of the agar slant stock. The overlay is prepared conveniently as follows. Two phosphate stock solutions are made up in 0.5 per cent saline. These stock solutions are mixed as directed in Table I to obtain desired pH concentrations.

It has been our experience that the addition of 0.025 per cent of agar to the

overlay is beneficial, apparently as an aid in maintaining the reduced oxygen tension needed for *E. histolytica* cultivation. It was found undesirable to add a greater concentration of agar because the resulting viscosity of the fluid interferes with settling of starch to the bottom of cultures.

It is a convenience to prepare the overlay in liter Erlenmeyer flasks provided with a siphon and test tube filling shield. The overlay is autoclaved and cooled before adding it to the slants. In a considerable series of tests, we have found no apparent difference in culture response between addition of overlay to fresh slants and addition to slants stored in the icebox for as much as two weeks. As a routine procedure, we have found it convenient to add overlay at the time tubes are inoculated.

The medium works well over a wide pH range, but for routine purposes, we have selected pH 7.4 for slant and overlay. Tests will be described later.

TABLE I
Buffered 0.5 Per cent Saline

M/30 Na ₂ HPO ₄ 4.75 GM. PER LITER	M/30 KH ₂ PO ₄ 4.53 GM. PER LITER	pH
cc.	cc.	
97.5	2.5	8.3
95.0	5.0	8.0
92.0	8.0	7.8
88.0	12.0	7.6
82.0	18.0	7.4
73.0	27.0	7.2
62.0	38.0	7.0
50.0	50.0	6.8
37.0	63.0	6.6

4. *Rice starch.* Rice starch or rice flour, sterilized in a hot air oven at 150°C. for 90 minutes, is added to the medium at the time of inoculation. The equivalent of a bacterial loopfull is adequate for a feces-inoculated culture, but two to three times this amount is appropriate for later sub-inoculations.

CULTIVATION PROCEDURE

Inoculation procedure. In diagnostic use of the medium, best results have been obtained by introducing a pea-sized lump of feces with an applicator stick, or with a pipette if the feces are liquid. The fecal mass need not be emulsified, for trophozoites or excysting organisms will leave it and accumulate at the bottom of the tube. A subculture after 24 to 48 hours incubation is a good routine procedure. All of the human parasitic amoebae respond in the "A" tube; in the "B" and later subcultures, *E. histolytica* usually responds vigorously, but the other species tend to die out.

Strain maintenance in culture. Alcoholic extract medium cultures are characteristically long lived, usually continuing in good condition for at least 10 days. Subcultures are usually made on the fourth to tenth day, but have been made

successfully as late as the seventeenth day. For sampling and subculture, we prefer one cc. pipettes of the type used for milk sampling. They have a larger bore than serological pipettes and the outside diameter fits a medicine dropper bulb. In the sampling of cultures, the pipette is used without bulb, the finger serving to open and close the top of the pipette in the process of drawing samples. In making subcultures, a bulb may be attached to the pipette to permit forcible stirring of sediment.

It has been our experience that small inoculi are preferable to large for subculture. In flourishing cultures showing saturation of the sediment at the bottom with amoebae, a tenth cc. of the stirred sediment is adequate for subculture. If the number of amoebae is very small, or if a blind passage is being made, we may transfer as much as a half cc. All our culturing to date has been done in 15 mm. x 150 mm. test tubes. The population limit which can be obtained from this size culture appears to be about 300,000. In the best cultures, the sediment at the bottom is saturated with amoebae and microscope preparations will show clusters with 150 to 200 amoebae in a high power field.

It should be pointed out that although large numbers of amoebae congregate at the bottom of the tube in the sediment, many take to the slant. We have made direct microscopic observation of amoebae on the slant by affixing the tube to the stage of a tilted microscope. Actively moving amoebae have been observed on the slant in this way within 15 mm. of the surface of the fluid overlay.

OBSERVATIONS

In our studies, we have used a number of strains of *Endamoeba histolytica*. These strains were isolated and kept under continuous cultivation for periods up to six months. In the course of these studies, we have obtained information on different types of extracts and basic procedures of preparation and use. Information relative to practical application of the medium has been obtained from its use in intestinal protozoan parasite surveys, and stool culture for *Endamoeba histolytica* diagnosis.

Medium Studies

Comparative culture series were run to obtain data on different types of medium. These series were evaluated by study of the population developed. Populations were estimated by count of organisms per low power microscope field, in a drop of culture sediment, and were recorded as + (if less than 5), ++ (5 to 20), +++ (20 to 40) and ++++ (over 40).

Extracts tested. A list of the extracts we have prepared and tested follows:

1. Human liver—four separate lots obtained at autopsy. One lot was used for over two years.
2. Calf liver—One lot purchased in a market and used for two years.
3. Beef liver—One lot purchased in a market.
4. Guinea pig liver—Two separate lots from laboratory guinea pigs. One lot was used for over two years.
5. Cat liver—Two lots from laboratory cats. One lot was used for over two years.

6. Cat intestine—Two separate lots. The small and large intestine were split and the contents removed before tissue preservation.
7. Egg yolk—Fresh eggs were broken, the yolks drained of white and dropped into 95 per cent ethyl alcohol in the same proportion as used for tissue.
8. Egg white—Egg white from fresh eggs was added to 95 per cent ethyl alcohol in the same proportion as used for tissue.

A standard 10 per cent extract was prepared from each of the above lots and in addition, a number of other concentrations. One lot of human liver was divided and 20 and 50 per cent extracts also prepared. One lot of beef liver, one of egg yolk and one of egg white were also prepared as 20 per cent solutions.

Basic tests relative to preparation procedure will be discussed first. In certain instances, we have been faced by two or more alternatives. For example, the question arose as to whether the agar-extract slant could be prepared by adding the alcoholic solution directly to the agar and subsequently eliminating the alcohol by cooking. In a number of tests, slants so prepared gave poor growth. We tentatively conclude that there is an advantage, possibly even a beneficial change, derived from the procedure we have adopted for preparing the alcohol-free extract before combining it with the agar.

Hydrogen-ion concentration. The phosphate buffered 0.5 per cent saline adopted as the fluid phase of the medium provides for a wide range of choice of pH. Groups of cultures were run in parallel at pH 6.8, 7.0, 7.2, 7.4, and 7.6. The liver extract media were found to give good growth in 7.0, 7.2, 7.4 and 7.6 with the best growth at 7.4. Egg yolk medium gave best growth at 7.6. At pH 6.8, the liver extract media gave fair growth, and the egg yolk extract medium, poor growth.

Extract concentration. One cc. of 10 per cent alcoholic extract per culture was adopted as standard. The resultant extract concentration per culture is about one per cent. This amount was selected after testing various concentrations. Cultures prepared from a 50 per cent human liver extract at the rate of one cc. extract per culture gave a tremendous bacterial growth but a very small and fleeting amoeba growth. The extract concentration in these cultures was about 5 per cent. Even this nutrient level seems low compared to the 10 to 20 per cent serum usually used in serum containing media.

Medium prepared from 10 and 20 per cent beef liver extracts, one cc. per culture to give one and two per cent media, gave 93 per cent +++ or better cultures for each extract. The one per cent extract, however, gave 30 per cent more ++++ cultures than the two per cent medium. In test series in which 20 per cent beef extract was used in one cc. and one-half cc. amounts per culture, the cultures prepared from one-half cc. amounts gave more ++++ cultures. The per cent of +++ or better cultures was about the same for the two media. Little or no amoeba growth was obtained by 0.1 per cent extract medium tests of calf liver, cat liver and guinea pig liver. 0.3 per cent extracts proved capable of supporting continued cultivation, but few cultures achieved +++ growth. Calf liver gave a slightly higher per cent of ++++ cultures at 0.7 per cent extract concentration than at 1.0 per cent.

On the whole, the dilution studies showed that a one per cent extract concen-

tration is near optimum. At about two per cent, increased bacterial growth is invited and the possibility remains that at higher concentrations the extract may inhibit amoeba growth. It would appear, however, that at the one per cent concentration, the low bacterial growth afforded by the low nutrient level is an important factor in promoting the long life of the cultures. The cultures remain relatively free of turbidity, gas production and odor production.

Autoclaved slants. We carried out several hundred tests to determine what effect autoclaving the tubed agar extract mixture before slanting might have on the potency of the medium. These tests have shown quite clearly that no apparent adverse effect or improvement results. Parallel series of autoclaved and unautoclaved slants of guinea pig liver, cat liver, calf liver, beef liver, human liver and egg yolk extracts have given uniformly about the same percentages of cultures achieving +++ and ++++ growth. Routinely, we prefer to prepare the agar-extract slants with aseptic precautions and have had no difficulty obtaining sterile slants by this procedure.

Extract comparisons. The variety of extracts which have been prepared and tested to date is small, but the results indicate that a wide variety of tissues from a wide variety of sources may be used successfully. It remains for future critical studies to determine whether there are consistent differences between extracts from different types of tissues or tissues from different species of animals.

Of the extracts we have tested, only egg white has given disappointing results. In parallel culture series with other extracts, egg white cultures seldom give a +++ culture. Further subculture to new egg white tubes usually results in the disappearance of amoebae by the third subculture.

The egg yolk extracts we have prepared have given consistently excellent results and impress us as being somewhat more productive of very high populations than the tissue extracts.

The liver extracts have all given approximately the same per cent of cultures achieving +++ or better growth and a similar length of productive life.

It is noteworthy that the cat intestine extracts have given results approximating those with liver. In each instance, several hundred cultures have been observed in evaluating the extracts.

In one test series, calf liver and egg yolk extract were mixed, half and half, and cultures prepared. In a parallel series of tests carried out at pH 7.6, the calf liver proved slightly inferior to the egg yolk and the mixture proved intermediate. It remains to be seen whether a nutrient complex, superior to the separate members of a mixture, can be produced.

Several lots of stock extract have been kept in the laboratory and used for a period of over two years. No apparent loss of ability to grow *E. histolytica* has appeared in this period.

Survey and Diagnosis

Alcoholic extract medium has been tested for the past three years in surveys and under routine laboratory conditions as a diagnostic cultivation medium.

In a parasite study of 107 Japanese Prisoners of War, we inoculated parallel cultures of alcoholic extract medium (human liver) and of liver infusion agar

medium (Difco Laboratories). The results of this series are given in Table II. Cultures were counted as positive if active trophozoites were found at 24, 48, 72 or 96 hours. The first column of the table gives the total results obtained by examination of fresh smears and zinc sulfate flotations from the stools.

A large factor in the relatively poor response of the liver-infusion-agar cultures was the development of heavy *Blastocystis* growth. This organism does not

TABLE II
Results Obtained from Examination and from Culture of Stools

ORGANISM	STOOLS POSITIVE BY EXAMINATION	POSITIVE ALCO- HOLIC EXTRACT MEDIUM CULTURES	POSITIVE DIFCO MEDIUM CULTURES
<i>Endamoeba histolytica</i>	8	10	7
<i>Endamoeba coli</i>	13	13	3
<i>Endolimax nana</i>	9	11	4
<i>Iodamoeba williamsi</i>	4	4	0
<i>Dientamoeba fragilis</i>	1	1	0
<i>Trichomonas hominis</i>	4	11	7
<i>Enteromonas hominis</i>	0	1	0
<i>Chilomastix mesnili</i>	0	1	1
<i>Giardia lamblia</i>	3	0	0
Totals.....	42	52	22

TABLE III
Number of Infections Found by Each Method of Examination

PROTOZOAN	INFECTIONS FOUND	SALINE AND IO- DINE PREPARA- TIONS	ZINC SULPHATE FLOTATION	HEMATOXYLIN STAINED PREP- ARATION	CULTIVATION
<i>E. histolytica</i>	2	1	1	2	2
<i>E. coli</i>	8 (3)*	5	5	7	8
<i>E. nana</i>	11 (1)*	4	7	8	10
<i>T. hominis</i>	1 (1)*	1		1	1
<i>E. hominis</i>	1 (1)*			1	1
Total.....	23	11	13	19	22

* Trophozoites only.

grow in the alcoholic extract medium cultures. The alcoholic extract medium cultures developed a relatively light turbidity, evidence of low bacterial growth compared to that in the liver-infusion-agar cultures. The liver-infusion-agar cultures seldom lived more than 48 hours, while the alcoholic extract cultures lived for about a week. *Giardia lamblia* did not grow in either medium. The positive "A" cultures of *E. histolytica*, *Trichomonas hominis*, *Enteromonas hominis* and *Chilomastix mesnili* gave good "B" subcultures. Amoebae other than *E. histolytica* gave little growth response in "B" cultures.

In a protozoan parasite survey of 100 healthy troops in the United States, stools were examined by saline and iodine stained direct film, zinc sulfate flo-

tation, iron-hematoxylin stained film and cultivation in alcoholic extract (human liver) cultivation medium. Table III gives the total infections found and the results from each examination procedure. The cultures revealed all but one of the 23 infections found by the other three procedures combined. A number of the infections recorded for the hematoxylin stain procedure were found only after prolonged search following appearance of the organism in culture.

SUMMARY AND CONCLUSIONS

Alcoholic extract cultivation medium has been tested for three years and found practical and effective as a medium for diagnosis of *Endamoeba histolytica* infection by feces culture as well as for the continued maintenance of *E. histolytica* strains in culture.

The basic ingredient of alcoholic extract medium is extract derived from tissue or egg yolk preserved in 95 per cent ethyl alcohol. A standard 10 per cent stock solution is made by adding 100 cc. of tissue or egg yolk to 900 cc. of alcohol. Medium is prepared for ten tubes by placing 10 cc. of alcoholic extract in a flask, driving the alcohol off by heating on a water bath, then adding 20 cc. of melted solution of two per cent agar in buffered 0.5 per cent saline, tubing the agar extract mixture in two cc. quantities and slanting, then adding an overlay of buffered 0.5 per cent saline to cover the slants. The medium is then ready for inoculation. No serum supplement is needed.

Extracts were prepared and tested from human liver, calf liver, beef liver, guinea pig liver, cat liver, cat intestine, egg yolk and egg white. Of these extracts, only egg white proved ineffective.

Tests over a pH range of 6.8 to 7.6 gave best results at pH 7.4 for tissue extracts and 7.6 for egg yolk extract.

Tests of various concentrations of extract indicate that the optimum is one cc. of the 10 per cent stock alcoholic extract per culture. This rate gives each culture the extract from approximately 0.1 cc. tissue (about 0.1 gm.).

Autoclaving the agar extract mixture before slanting does not appear to affect the medium.

Tests to compare media prepared from the different extracts gave about the same results for all the tissues and egg yolk. A mixture of egg yolk and calf liver extract gave approximately the same results as the extracts separately.

In a protozoan parasite survey, alcoholic extract medium gave results superior to those obtained by parallel cultures of liver-infusion-agar. Alcoholic extract medium appears to have a good ability to develop positive cultures from small numbers of organisms in feces. To some extent this ability is enhanced by the fact that the medium does not grow *Blastocystis* or a very heavy bacterial population.

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THE METABOLISM OF CHINIOFON USING RADIOACTIVE IODINE¹

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INTRODUCTION

Chiniofon, an organic compound containing approximately 27.5% iodine by weight (fig. 1), has been used in the treatment of amebiasis for many years. Despite continued clinical use little is known of its fate in the body. Speculation has provided most of the information concerning its metabolism. Certain authorities (1) state that it is not absorbed and hence acts solely within the intestinal tract, while others (2) believe that it is possibly absorbed and acts both through the blood stream and bowel. Our interest in this question stemmed from wartime experience with amebiasis among troops in Pacific islands, where chiniofon was used extensively, despite such divergence of opinion regarding its distribution in the body.

The purpose of this study was to investigate the fate of chiniofon in the body. Specifically, it was desired to determine, first, whether absorption does occur, and if so to what extent and at what rate; second, if absorption occurs, is it sufficient to develop blood levels of clinical importance; and third, the pattern of possible urinary excretion.

METHODS AND MATERIALS

The availability of radioisotopes has made possible the use of the tracer technic in this investigation. Stable iodine ordinarily occurring in the compound (fig. 1) was replaced with iodine-131 having an eight day half life. Radiochiniofon, like the stable form, is easily soluble in water with the addition of a small amount of sodium bicarbonate, forming the sodium salt as it goes into solution. The compound is bright canary yellow in color and has a bitter taste.

The radioactive iodine was prepared by the bombardment of powdered tellurium, once in the Washington University cyclotron, and once in the Oak Ridge pile. The metal presumably containing 80 mc. of activity was dissolved in hot 30 per cent sulfuric acid (300 cc.) in a liter 3-necked flask by the gradual addition of solid chromic acid. When a permanent green color persisted, 9.0 gm. of potassium iodate was added, and then, cautiously, an excess of oxalic acid. The flask was connected to an efficient bulb condenser and trap. On warming the liberated iodine gradually distilled and was kept from clogging the condenser by the slow addition to the distilling flask of chloroform. The distillate was reduced by SO₂, the chloroform and excess SO₂ removed by warming and the liquid neutralized by calcium carbonate.

¹ This work was supported by a grant from the Wisconsin Alumni Research Foundation.

The somewhat concentrated solution was treated with 8.6 gm. of 8-hydroxyquinolin sulfonic acid, followed, during boiling, by a solution of 3.6 gm. of "HTH" (calcium hypochlorite). After cooling dilute HCl was added over several hours with good mixing, avoiding an excess of liberated iodine. Purification was carried out by dissolving in Na_2CO_3 acidifying strongly by HCl, and washing well with alcohol. Yield: 10-11 gm.

Instrumentation

The equipment used included a bell-jar type Geiger-Muller counter tube having a mica window sufficiently thin to permit counting beta radiation of I-131. The tube was mounted vertically on a wooden rack, and samples were placed beneath its window on copper discs supported on an adjustable sliding vernier. This arrangement afforded completely reproducible geometry. A lead shield for the tube, regulated high voltage supply, scaler and mechanical register completed the assembly. A uranium glass standard giving a long-lived source of

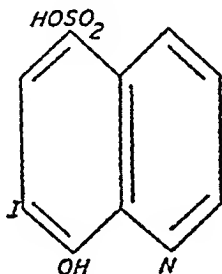


FIG. 1. STRUCTURE OF CHINIOFON

radioactivity was used before and after each session of counting to determine the reproducibility of the entire system. Background counts were taken before each counting session and remained close to 25 counts per minute. For computation of data a known weight of radiochiniofon was counted at the end of each session.

Subjects

Seven individuals were used as subjects. They varied in age from 30 to 65 years, two were female and five male, five of them were hospitalized for various conditions (mild diabetes, arthritis, Hodgkin's disease) although they were ambulatory and able to cooperate. Two of the subjects were healthy individuals. None had amebiasis.

Administration

The drug was given in an aqueous solution of its sodium salt, orally in single doses, ranging in amount from 100 mg. to 300 mg., depending on the intensity of the radioactivity contained. Care was taken never to exceed 0.5 millicurie per person. The test dose was taken at 7:00 A.M. in the fasting state, following which the subjects were permitted their usual food and fluid intake.

Collection of Specimens

Five cc. samples of blood were drawn into anticoagulant. In one subject blood was obtained at 2, 4 and 8 hours and in two subjects at 1, 3, and 6 hours respectively after ingestion of the drug. Urine was voided and discarded immediately before taking the drug. Following this all urine was saved in three hourly collections for the first 12 hours, then in a single 12 hour collection and lastly a single 24 hour collection, making a total of 48 hours. All stool specimens were saved for a period of 7 days, being collected directly in cardboard containers of known weight.

Preparation of Specimens

In the case of blood a protein-free filtrate was used in order to reduce beta ray absorption. This was obtained by mixing 5 cc. of whole blood and 4 or 5 parts of 2.5 percent trichloroacetic acid and filtering. 0.5 cc. of the filtrate, corresponding to 0.1 or 0.08 cc. of original blood respectively, was measured on to the copper disc for drying and counting. Samples were run in duplicate. Urine was processed in two parts. A known volume of raw urine was diluted with distilled water in a volumetric flask and 0.5 cc. of the mixture corresponding to 0.1 cc. of original urine was measured on to discs for drying and counting. This gave a measure of the total radioactivity present in a specimen, that is, the sum of radioactivity from chiniofon-bound iodine and free split iodide. Another aliquot of urine was taken through a procedure designed to remove all free iodide present and leave only iodine bound to chiniofon. This served as a measure of chiniofon being excreted in the urine, and when compared with the total radioactivity present gave an indication of the extent to which the body split the drug into iodide and organic residue. This was done by acidifying 10 cc. of urine with 1 cc. of concentrated nitric acid to keep silver-chiniofon complex in solution, adding excess of 0.1 normal silver nitrate (usually 20 cc. sufficed), diluting with distilled water in a volumetric flask, and filtering off the precipitated silver iodide and silver chloride. Chiniofon and/or its silver complex remained in the filtrate, an amount of which to correspond to 0.1 cc. of original urine was dried and counted. Both procedures were run in duplicate. Extremely thin films were obtained when the samples were dried. The stool specimens were dried by placing in an electric oven with temperature of 85-90°C. for 24 to 48 hours. The dry weight was obtained and the entire stool was powdered and thoroughly mixed to insure even sampling. A 5 mg. aliquot was then weighed on to copper discs in duplicate and spread evenly over an area approximately 1 to 1.5 cm. in diameter by means of a couple of drops of ether followed by water and then dried. All fluid measurements were done with standard 1 cc. tuberculin syringes.

RESULTS

Blood

In figure 2 is shown a composite curve of blood levels of chiniofon following a 100 mg. dose in each of two subjects. The radioactivity as gammas of radio-

chinfofon per 100 cc. of blood is plotted against time in hours. The levels shown are 27 gammas at one hour, 190 at two, 110 at three, 60 at four, 50 at six, and 30 at eight hours. This indicates that the blood level builds up sharply to a peak within two hours and then falls off smoothly during the next six hours. If it is remembered that a variable fraction of this radioactivity represents free iodide and the rest chinfofon-iodine, as will be revealed in the urinary data, there is no confusion in interpretation. In this connection it is of interest to note that a survey over the thyroid gland in two subjects gave approximately 0.06 milliroentgens per hour of radiation the day following ingestion, with none detectable three days later. Such activity is presumably due to free iodide.

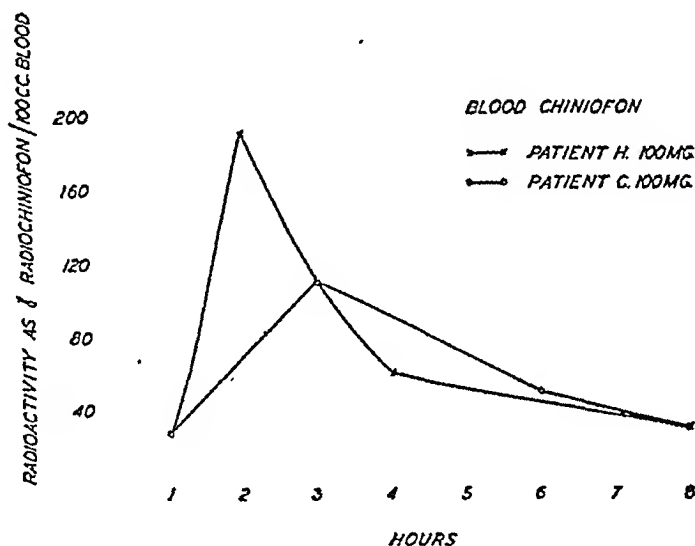


FIG. 2. A COMPOSITE CURVE OF BLOOD LEVELS OF CHINFOFON FOLLOWING 100 MG. DOSE IN EACH OF TWO SUBJECTS

The radioactivity as gammas of radio-chinfofon per 100 cc. blood is plotted against time in hours.

Urine

Urinary excretion has proved to be strikingly similar in all seven subjects. In each case there is a high initial level of excretion during the first three hours, which then falls abruptly during the first twelve hours following ingestion of the test dose. During the last thirty-six hours excretion tapers off gradually to the vanishing point. (Urinary excretion does continue into a 72-hour period, but in the two subjects tested the additional amount was found to be so small and in line with the established curve that further observations on urine were not carried beyond the 48 hour period.) In figures 3 and 4 are shown representative curves of urinary excretion, in which counts per minute in millions are plotted against time in hours. In these charts the upper curve shows the total radioactivity on the raw urine, while the lower curve represents the radioactivity from iodine bound to chinfofon. The total radioactivity appearing in the urine in 48 hours in the 7 subjects has varied from 7.46 per cent to 17.5 per cent of the administered amount, with an average of 12.9 per cent. The chinfofon-bound

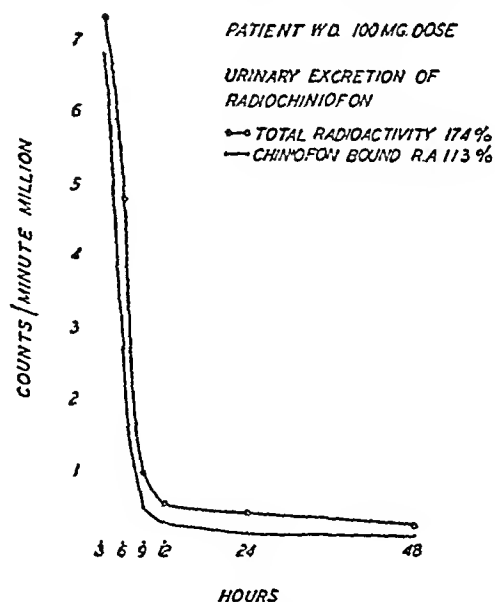


FIG. 3. A REPRESENTATIVE CURVE OF URINARY EXCRETION OF RADIOCHINIOFON FOLLOWING A 100 MG. DOSE

17.4% of administered radioactivity appeared in the urine, of which 11.3% was bound to chiniofon. The difference (6.1%) represents free iodide which was split from the drug during metabolism in the body.

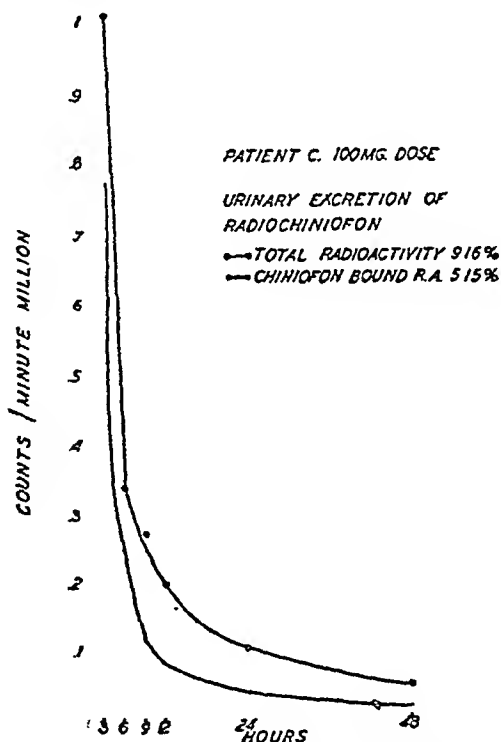


FIG. 4. ANOTHER REPRESENTATIVE CURVE OF URINARY EXCRETION OF RADIOCHINIOFON FOLLOWING A 100 MG. DOSE

9.16% of administered radioactivity appear in the urine, of which 5.15% was bound to chiniofon. The difference (4.01%) represents free iodide which was split from the drug during metabolism in the body.

activity has varied from 4.2 per cent to 11.3 per cent with an average of 7.4 per cent.

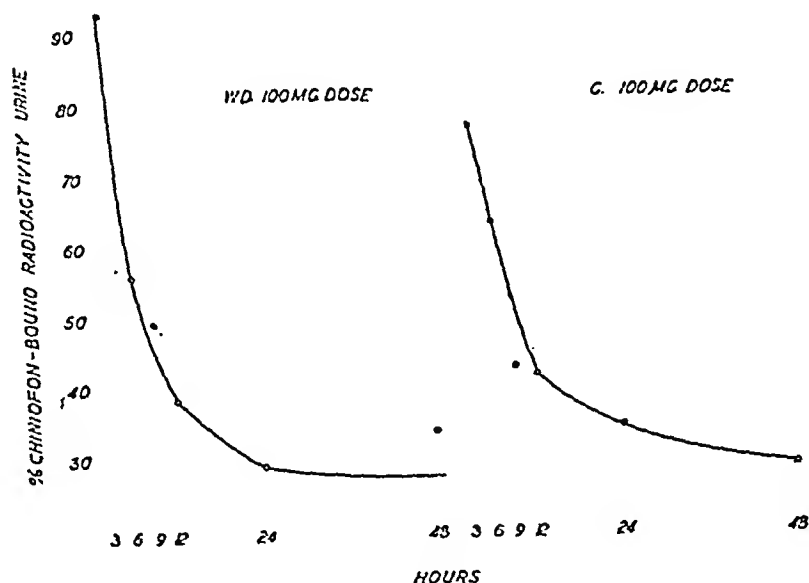


FIG. 5. CURVES SHOWING THE PROPORTION OF RADIOIODINE BOUND TO CHINIOFON DURING URINARY EXCRETION

At the beginning of excretion a high percentage of the radioiodine in the urine is bound to chiniophon, while as time elapses this percentage falls as a relatively greater proportion of iodine is split from the drug yielding free iodide plus organic residue. This was a constant feature of metabolism and excretion of the drug.

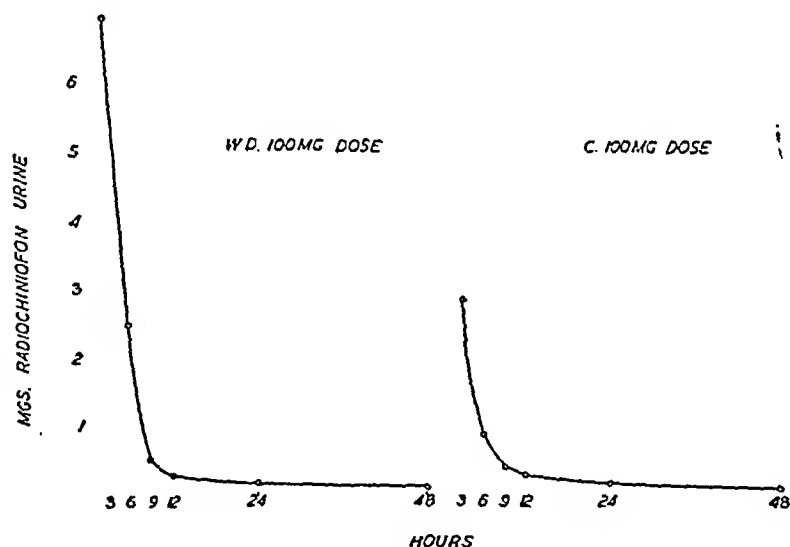


FIG. 6. CURVES ILLUSTRATING URINARY EXCRETION OF RADIOCHINIOFON IN MILLIGRAMS IN TWO SUBJECTS EACH GIVEN 100 MG. DOSE

W. D. totalled 11.3 mg. for 48 hours and C. 5.15 mg. for 48 hours. The 24 and 48 hour values are plotted on a 3 hour basis to provide comparability with the values for the first 12 hours of excretion.

Another constant feature of urinary excretion of the drug was the fraction of radioactive iodine split off from the drug as time elapsed. Figure 5 demonstrates

this clearly for the same two subjects. The percentage of chiniofon-bound radioactivity is plotted against time. At the beginning of excretion it will be noted a high percentage of the iodine is bound to the drug, and as time goes on this percentage falls as a relatively greater proportion of iodine is split from the ring to become free iodide. This pattern was observed in all subjects. The total percentages bound varied in the 7 subjects from 54 per cent to 66 per cent with an average of 58.6 per cent.

Figure 6 illustrates in the same subjects the actual weight of radiochiniofon in milligrams excreted over the 48 hour period, 5.15 and 11.3 mg. respectively. As indicated above the range has been from 4.2 to 11.3 mg. with an average of 7.4 mg. referred to a 100 mg. dose.

Stools

Fecal excretion has been found to persist for from 5 to 7 specimens, being related directly to elimination rather than time. In the last two subjects tested it was possible to recover from the stools 62.6 per cent and 52.1 per cent of the administered radioactivity. When added to the urinary recovery for these subjects a total recovery of 73.5 per cent and 69.65 per cent was found. Stool recovery on the first five subjects was lower, due probably to technical reasons.

DISCUSSION

As a result of these studies it is possible to state that absorption of chiniofon does take place. This is regular for it was noted in all of the subjects studied. The amount absorbed is small, averaging 12.9 per cent of the dose given, and as might be expected varied considerably, from 7.46 per cent to 17.5 per cent. This variation is conceivably due in part to individual fluctuations in the rate of passage of the drug through the intestinal tract. The absorption is prompt as shown by the blood and urinary findings. A peak blood level in two hours following ingestion, and the occurrence of the highest urinary excretion during the initial three hour period testify to this.

Urinary excretion of the drug has been found to occur in a similar pattern for all seven subjects. It is rapid, the bulk being eliminated by 12 hours, and virtually completed in 48 hours. Excretion of the drug is further characterized by its appearance initially in the urine almost entirely intact (averaging for the first three hour period 82 per cent intact as shown by chiniofon-bound iodine and 18 per cent split off free iodide and residue as shown by the difference between total and bound radioactivity) and subsequently reversing this ratio in the second 24 hour period to appear largely split (averaging 27 per cent intact and 73 per cent split). For this reason the amount of intact chiniofon excreted is less than that absorbed, and varied from 4.2 per cent to 11.3 per cent of the administered dose, averaging 7.4 per cent. Approximately 58.6 per cent (average) of excreted radioactivity is from chiniofon iodine, the rest being free iodide and organic ring.

It is believed that fecal excretion plus urinary excretion should equal the total dose of drug given. The inability to obtain a total recovery higher than 73

per cent rests probably on technical difficulties involving fecal analyses, and does not imply that storage of the drug occurs. It has been shown that such is not the case in the thyroid gland.

Blood levels of clinical importance can not be attained even with rapid absorption of the drug, because of the uniformly low percentage of absorption, the rapid urinary excretion, and the body's breakdown of the drug into free iodide and organic ring.

CONCLUSIONS

Following the administration of a single dose of radiochiniofon to seven subjects it has been observed that:

1. Absorption of the drug occurs regularly, is small in amount averaging 12.9 per cent of the dose given, and is rapid with a peak blood level occurring in approximately two hours.

2. A consistent pattern of urinary excretion occurs, with the bulk appearing in the first 12 hours and being virtually complete by 48 hours.

3. Chiniofon is partly broken down after absorption as a progressively greater proportion of iodine is split off from the compound. During the initial period of observation the drug is excreted 82 per cent (average) intact, while at the end of observation only 27 per cent is intact. For the entire 48 hour period 58.6 per cent (average) of the drug excreted in the urine is intact chiniofon, the balance being free iodide and organic residue. A portion of the free iodide fraction is detectable qualitatively in the thyroid gland in situ.

4. The unabsorbed portion of the drug appears in the feces, from 5 to 7 specimens being required for complete elimination.

5. Blood levels of clinical significance are not attainable with doses used.

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TYPES OF AMERICAN CUTANEOUS LEISHMANIASIS— DERMATOLOGICAL ASPECTS

A REVIEW

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Received for publication February 25, 1947

Cutaneous leishmaniasis is of clinical interest to American dermatologists and now even to those in North America, because of the frequency of this infection in many areas of tropical and subtropical America, its appearance during the past war in military forces of the United States, and the occasional cases seen in large centers of dermatology in the United States. Moreover, cutaneous leishmaniasis has a number of important features for those who are concerned with immunobiology of the skin. Through efforts of Peña Chavarría and his associates in Costa Rica, and Latapi and Gutiérrez and Beltrán in Mexico and Pedro Weiss (1) especially, Loret de Mola and Arana in Peru, clinical material was made available. The author has had no personal clinical experience with cutaneous leishmaniasis in Venezuela or with the vast amount of material and with the extensive investigative work in Brazil. Shattuck (2) in 1938 claimed that the cutaneous forms of leishmaniasis differ considerably in appearance and severity, and the different types tend to be distributed in regions. Fox (3, 4) in 1934 reviewed the general aspects of American leishmaniasis and emphasized the desirable elimination of local sectional names which serve to confuse the literature. It is the opinion of the author also that the clinical picture of primary cutaneous leishmaniasis does vary even in the relatively few areas where he has made observations. Many excellent reviews of the clinical forms of American cutaneous leishmaniasis have been given by Weiss, Monge, Escomel, Puente, Rabello, Tamayo, Peña Chavarría and others.

Cutaneous leishmaniasis, of course, may start as a primary cutaneous disease following inoculation with *Leishmania*, or cutaneous involvement may occur during the course of a visceral leishmaniasis. In endemic areas of cutaneous leishmaniasis I saw cases of suspected visceral leishmaniasis, but the clinical picture was confusing, the cause of the visceral involvement was not proven microscopically, and the therapeutic response to antimony salts was so indefinite, a combination of circumstances taken as proof that these were cases with nonspecific (avitaminotic?) type dermatitis. However, even in relatively isolated communities, in South America, the co-existence of cutaneous leishmaniasis, primary type, and of an occasional case of kala-azar appears to have been proved. Our interest, then will be chiefly in the primary cutaneous leishmaniasis.

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From a clinical aspect it is possible to build a basic structure for the cutaneous changes in cutaneous leishmaniasis, American or otherwise. Thus:

Inoculation \rightarrow papule (s) \rightleftharpoons ulcer (s) \rightarrow scar (s)

The more common possibilities for individual variation in each portion of the basic structure may be listed:

I. Inoculation:

- A. "usual" insect bite type
- B. petechial
- C. insignificant
- D. no evidence (?)
- E. mucosal (??)

II. Papule (s):

- A. conventional papule type
- B. nodule or node—superficial or deep
- C. excessive acanthotic changes—verrucous or frambesiform
- D. mucosal changes—papular or markedly acanthotic

III. Ulcer (s):

- A. cutaneous—superficial or deep
- B. mucosal

IV. Scar (s):

- A. healed
- B. active foci—infection or allergic (leishmanid)

The basic structure of cutaneous leishmaniasis secondary to visceral involvement, appears to be:

a) cutaneous inoculation \rightarrow visceral involvement \rightarrow cutaneous involvement
 \searrow
mucosal involvement

b) cutaneous involvement \rightarrow skin negative clinically
 \rightarrow papulo-nodular
 \rightarrow pigmentation
 \rightarrow ulcerative (uncommon)

c) mucosal involvement \rightarrow mucosa negative clinically
 \rightarrow ulcerative
 \rightarrow acanthotic

The clinical pictures of primary cutaneous leishmaniasis observed in a geographical region, especially if it is an endemic area, are simply variants of the

basic structure of the pathogenesis of the infection. There is much which is purely speculative about the reasons for these changes which will be mentioned later, as the true causes are not known. For clinical purposes, a simple general classification of primary American cutaneous leishmaniasis may be listed.

- I. Cutaneous
- II. Mucosal:
 - A. primary
 - B. metastatic
- III. Mixed

For more detailed emphasis of the clinical aspects of the variants of the basic structure there is the classification of Senckejeie (5).

- I. Old world cutaneous type:
 - A. abortive type, small
 - B. regular single oriental sore
 - C. multiple type
 - D. lymphangitic type
 - E. verrucous type
 - F. lupus leishmaniasis
- II. Muco-cutaneous American type:
 - A. primary and metastatic, uta, espundia
 - B. chiclero ulcer type

In Central and South America the detailed and excellent clinical classification of Rabello (6) is preferred.

- I. Cutaneous:
 - A. ulcerative
 - 1. impetiginoid
 - 2. ecthymatoid
 - 3. true ulcer
 - B. non-ulcerative
 - 1. nodular dermal
 - 2. vegetant
 - a. frambesoide
 - b. verrucous
- II. Subcutaneous ("pro parte"):
 - A. non-ulcerative
 - 1. nodular hypodermal
 - B. secondary ulcerative ("nodular ulcerada")
- III. Mucosal:
 - A. vegetant—non-ulcerative
 - B. vegative—ulcerative

- IV. Mixed: includes various combinations of the above

No opportunity was afforded for the study of the inoculation mechanism of American cutaneous leishmaniasis. The *Phlebotomus* is blamed but it appears, at least in Mexico, Central America and Peru, that its rôle has not been established definitely. Hertig has made extensive studies on the *Phlebotomus* in

Peru and also epidemiologic surveys, and with Herrer and Battistini has studied the incidence of leishmaniasis in Peru. The close regional relationships of the endemic foci of leishmaniasis and of human bartonellosis in Peru is a singular feature of the pathology of this country.

Direct contact infection of the skin and secondary leishmania infection of skin lesions may occur, and perhaps if controlled observations were available, this mechanism of local infection may be more common than is generally supposed. At least with Old World cutaneous leishmaniasis (9), the incubation period is long, weeks to several months. With experimental infections, however, with large numbers of living flagellates (Dostrovsky) (7), erythema-oedema lesions may appear within twenty-four hours following intracutaneous inoculation. The role of animal reservoirs of infection also has not been established critically as yet in the Americas. In Mexico even *Simulium* and *Acarus* have been suspected as possible vectors. In Guatemala, Padilla (8) has tried xeno-diagnosis with *Triatoma*, with negative results. The *uta* form (usually facial ulcerative) of leishmaniasis in Peru has been suspected to follow bites of insects long before the discovery by Wenyon in 1911 of the *Phlebotomus* vector of Old World cutaneous leishmaniasis. A keen observer, Brother Diego de Morales cited by Palma, referring to certain areas of the Andes where infections about the nose occur reported in 1602 that: "all there become ill with a sore from the mosquito." Although a number of patients offered a history of "pimple" as their primary lesion, such lesions, proved bacteriologically, were not observed in Mexico, Costa Rica, or in some of the villages of the province of Huarochiri in Peru. Although the classification form, list many variants, certain lesions are more common. The more common type appears to be lesions on exposed surfaces, especially the ear in Mexico, ear (figures 1 A, B, C) and forearms and then leg in Costa Rica, and face in Peru. Although detailed descriptions have been given of the character of the ulcer base and type of crust, etc. it is felt that to the inexperienced observer there is nothing distinctive about these ulcers. The "Botón de Vélez" of Venezuela has been described by Gutiérrez (10) as having a black fetid crust. Many of them (true ulcer of Rabello) would be suspicious of late syphilis in the temperate zones or in military medicine, of ulcerative diphtheritic lesions. The ear lesions, in their well developed phase, resembled ulcerative carcinomas. In Peru, the early face lesion resembled oriental sore (figures 2 A, B, C, D). This resemblance has been noted in Peru as far back as 1852 by José Julian Bravo. Fox (3, 4) has also noted this similarity. In Peru, cutaneous leishmaniasis, especially the *uta* type, is much more common in certain regions of the Cordilleras, as described by Ugaz (12), "en los espacios intercostales de la gran columna vertebral de los Andes." In fact Weiss considers that in extent of distribution, cutaneous leishmaniasis ranks next to malaria in Peru. Strong, Hertig and Herrer have studied the incidence of this *uta* form in Peru. Even in the brief experience of the author, it was not uncommon to stand in the main street of small towns in some sections of the province of Huarochiri, and see small facial scars on almost every child and in adults, large mutilating scars on the face, and occasionally one with amputation of the nose (figure 2B). Urica

claimed that infancy, adolescence and youth are most afflicted with the uta, and Palma said the majority of cases occur before four years of age.

In Peru the facial lesions were as a rule, especially in the adult, much more extensive than in the child, with perforations into the buccal cavity and direct



FIG. 1. THE EAR LESIONS IN AMERICAN CUTANEOUS LEISHMANIASIS (CHICLERO ULCER)

FIG. 1A. Costa Rica, there was an associated leg ulcer (fig. 3B)—courtesy Dr. Arturo Romero.

FIG. 1B. Mexico.

FIG. 1C. Costa Rica.

FIG. 1D. Costa Rica, note the associated serpiginous ulcer of the forearm.

extension into the nose (primary mucosal) (figure 2 B). Ulcerative forms of cutaneous leishmaniasis on the covered (?) surface of the lower back have been described by Costa (13) in Brazil, and by Kuczynski-Godard (14) in the Peruvian selva. Occasionally in Latin America you hear the dictum that ulcers above the waist are leishmaniasis, those below, so-called tropical ulcers. This is not an

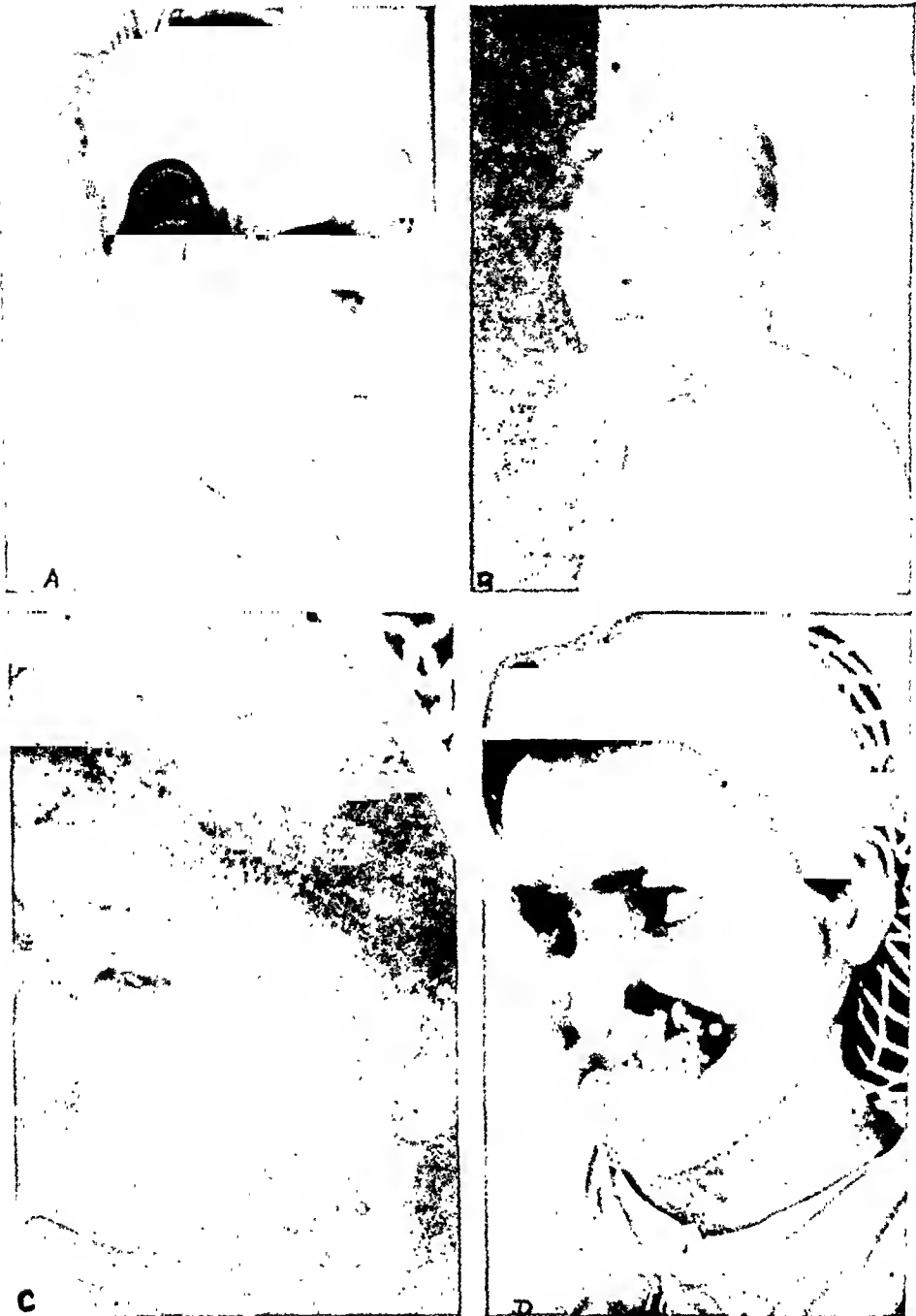


FIG. 2. TYPES OF FACIAL LESIONS IN PERU

FIG. 2A. Typical ulcerative form with almost complete scarring.

FIG. 2B. Old scarred lesion of the face commonly observed in villages in the endemic zones (Province of Huarochiri).

FIG. 2C. Old ulcerative form with keloidal reaction, active foci still present in scar tissue, ulcerative lesions of the hands, also with keloidal reaction, healed.

FIG. 2D. Primary mucosal type of American cutaneous leishmaniasis from perforation of facial ulceration.

equivocal declaration even in those regions where most diagnosis are made by inspection alone, and tropical ulcer, especially in Latin America, is a type of cutaneous reaction of varied etiology, and not a single disease. In Costa Rica, a number of tropical ulcers of the middle and lower third of the legs, which types in the temperate zones would be in young people ecchyma or perhaps even ulcerative erythema induratum, and in older people stasis syndrome, were found to be due to leishmaniasis (figures 3 B, D). Moreover, stasis syndrome was



FIG. 3. TYPES OF LESIONS OF THE EXTREMITIES

FIG. 3A. Peru, keloidal reaction of ulcers of the hand (see fig. 2C).

FIG. 3B. Costa Rica (courtesy of Dr. Arturo Romero).

FIG. 3C. Costa Rica.

FIG. 3D. Costa Rica.

a frequent complication also, especially in chronic leishmania ulcers of the legs. Although vegetative forms are said to occur in the non-ulcerative types, yet vegetative lesions associated with leishmania ulcers of the extremities were seen. Leishmania leg ulcers occur less commonly in Panama and in Peru. Especially on the extremities and occasionally on the face, deep punched out "gummatous type" ulcers were found. This form and the serpiginous ulcerative types in the temperate zones would be characteristic, clinically, of late skin syphilis. At Puente Verrugas in Peru, a typical "gummatous" ulcerative lesion of the heel was observed. The spread of this was due to inadequate antimony therapy. In North America, leishmania leg ulcers are uncommon, chiefly because most of

the Old World type "imported" lesions are on the face. However, in the returned military personnel, active lower extremity lesions have been seen although most of the lesions occurred on the face and arms. Ordinarily differential diagnosis of chronic ulcerative lesions on the lower extremities in North America need not consider leishmaniasis. The disputed case of Stewart and Pilcher will be considered later. As Gutiérrez has indicated, no leishmania lesions of the palms, soles or scalp were noted. Aleixo (15) in Brazil and Weiss in



FIG. 4. SOME ATYPICAL FORMS

FIG. 4A. Costa Rica, ecthymatoid lesions of the chest.

FIG. 4B. Peru, "uta macho".

FIG. 4C. Peru, impetiginoid facial form.

Peru have reported genital leishmaniasis. These cases very much resemble our cases of genital *granuloma inguinale*. Opportunity was afforded to study one case of the impetiginoid form on the service of Arana in Hospital del Niño in Lima (figure 4C). The lesions were superficial, more impetiginous than ecthymatous. There was considerable adjacent erythema. No eozymatoid reaction was present. The residence of the child, presence of parasites and response to therapy were characteristic. An ecthymatoid leishmaniasis was seen in Costa Rica (figure 4B). An interesting and peculiar type of so-called dermal leish-

maniasis was the case of *uta macho* of Herrer and Weiss observed at Hospital del Niño in Lima. In this boy, the face showed criss-cross scarring with extensive elephantiasis (figure 4C). Such elephantiasic forms of *uta* had been described in 1875 by Antunez and Minaya in 1886. According to Rabello nodular dermal lesions comparable to the more common types of post kala-azar dermal leishmaniasis have not been observed in Brazil. In Matucana, a heavy endemic area, the author observed an interesting nodular leishmaniasis, across the bridge of the nose in a mestizo patient of early middle age. No laboratory examinations were made of this lesion. From a distance the lesion looked like a typical discoid lupus erythematosus which is observed not uncommonly in Peru. However, on close inspection the lesion was a deep nodular keloidal type lesion sans previous ulceration. Peruvian and Argentinian workers have reported a variety of nodular, papular and "papulo-tuberculosa" forms of cutaneous leishmaniasis.

Some cases of vegetative dermatitides of the lower extremities were seen in which leishmaniasis were suspected but not proved. Rabello has mentioned these types. The old term of *forest yaws* (*pian bois*) reveals how cutaneous leishmaniasis, especially in neglected cases, may resemble yaws. In none of the cases of cutaneous leishmaniasis observed, with and without therapy, were lesions suspicious of any form of so-called leishmanid observed.

One of the interesting forms of the deep cutaneous lesions are the so-called lymphatic leishmania nodules. These may occur primarily as such or the lesions may extend secondarily from an ulcer. In only one case of this type, presented as such, puncture studies were negative and biopsy revealed tuberculoid type reaction without organisms. Escomel in Peru first described this form in America.

It is the mucosal involvement which is so characteristic of American cutaneous leishmaniasis. The mucosal involvement may be of two types, primary from direct extension of cutaneous leishmaniasis of the facial area, and secondary or metastatic. Mucosal leishmaniasis was not observed in Mexico, and Gutiérrez states that it has not been seen in that country. This form of leishmaniasis appeared uncommon in Costa Rica, but was relatively common in Peru. In his report of cutaneous leishmaniasis in Panama, Kean (16) mentions one case of involvement of the naso-pharynx. In neglected or mistreated cases, chiefly by cauterization of the "folk-lore type", the ulcerative or other forms of facial leishmaniasis may spread with the development of noma-like lesions into the buccal cavity. Such extensive lesions appear more common in the adult. Monge has emphasized the more destructive nature of cutaneous leishmaniasis adjacent to cartilage of ear, nose and lips. However, ulcerative leishmaniasis, not nodular, overlying the bony bridge of the nose is rare. Leishmaniasis of the eyelids also causes considerable deformity.

The metastatic form of mucosal leishmaniasis is known in the selva by the Indian term of *espundia* (figures 5A, B, 6A, B, C). In Peru, at least, such metastatic types are rare, if present at all, clinically (as opposed to bacteriologically) in the *uta* regions. When more work is done with detailed examina-

tions of scrapings from "normal" nose for leishmania, then clearer understanding of mucosal leishmaniasis may be possible. Work of this type has been done in Brazil. The mechanism for the development of the metastatic form is not understood but some speculations regarding this will be discussed later. The metastatic lesions may coexist with distant cutaneous lesions or may appear some time, years, after the apparent complete healing of the primary cutaneous focus. Rarely, it may precede any cutaneous lesions or be even the only apparent manifestation of the leishmania infection. Weiss (1) gives figures for the frequency of mucosal involvement varying according to regions from twenty



FIG. 5. MUCOSAL FORMS

FIG. 5A. Peru, metastatic type, espundia (courtesy Dr. Pedro Weiss).

FIG. 5B. Peru, metastatic type, espundia (courtesy Dr. Pedro Weiss).

to eighty per cent. In a case observed in San José, Costa Rica, an old primary arm ulceration and an "metastatic" nasal lesion had long healed under antimony therapy when a leg ulcer, supposedly due to leishmaniasis relapsed. The cutaneous trauma factor in leishmaniasis has been noted before. It was not clear whether stasis factors or leishmania infection caused the relapse of the leg ulcer (figure 6C). The metastatic lesion in Peru, at least, affects the adult male usually. The mucosal lesions, start usually in the mucosa of the respiratory tract especially in the nasal septum. The primary lesion according to Klatz and Lindenberg, is not made by a superficial ulcerative process but by a pericapillary infiltration of the vessels of the sub-mucosa. Any portion of the respiratory tract may be involved, including the bronchi. In the mouth lesions may be found on the palate, cheeks and mucosal portions of the lips. Weiss (1) mentions

a tongue involvement which appeared very similar to a syphilitic glossitis. The mucosal lesions are essentially ulcerative or vegetative. Weiss (1) believes the palatal lesions are more commonly proliferative than destructive. Costa (13) has reported a nasal polyp type reaction and indicated that "this type of leishmaniotic polyp has not been recorded outside of Brazil." Emphasis has been



FIG. 6. MUCOSAL FORMS

FIG. 6A. Peru, metastatic type, espundia (courtesy Dr. H. Kuczynski-Godard, *La Vida en la Amazonia Peruana*, Libreria Internacional del Peru, S. A. Lima, 1944).

FIG. 6B. Peru, metastatic type, espundia (courtesy Dr. H. Kuczynski-Godard, *La Vida en la Amazonia Peruana*, Libreria Internacional del Peru, S. A. Lima, 1944).

FIG. 6C. Costa Rica, metastatic type, espundia, with nasal lesion and old elbow healed, later relapse of a leg ulcer.

laid upon the fact that the adjacent skin is intact, *i.e.*, "resists invasion." However, occasional ulcerations (due to leishmania?) may occur and Weiss calls these "lesiones terciarias de la Espundia." A rosaceal aspect (chronic passive congestion?) may be offered by the adjacent skin especially of the nose. Weiss claims that metastatic leishmaniasis affects only the cartilaginous not the bony portion of the nose. The general condition of the far advanced case may not be good because of interference with respiration and ingestion of food. Salivation may be excessive. Even in those lesions which eventually heal, considerable deformity may be produced by scarring.

The scars of cutaneous leishmaniasis may show either complete healing or occasionally, areas of still active foci (figure 2C). This is similar to the picture of the scar regions of lupus vulgaris. Careful diascopic pressure examination of scarred facial lesions especially in the adult is necessary. The leishmanid reaction about scarred lesions, has not been reported in American cutaneous leishmaniasis. The observations, in Old World leishmaniasis, of cutaneous tuberculosis developing in scars of leishmaniasis have not been reported in the Americas.

The clinical diagnosis of American cutaneous leishmaniasis is difficult for the inexperienced dermatologist unless the patient is seen in, or is reported to come from a frank endemic zone. The location on an exposed portion of the body, the chronicity of the ulcerative lesion and the response to antimony salts are, of course, some clinical aids in diagnosis. In the tropics, the therapeutic test with antimony salts is of only limited value. At least in Peru, in the endemic areas of *uta*, there are no other common types of skin disease which could cause similar forms of ulcerative lesions. Gummata, chronic pyodermias of ecthymatous and chaneriform types so-called tropical ulcer syndromes, cutaneous diphtheria and artefactual cutaneous lesions are some of the more common lesions which may offer difficulties. In returned military personnel in the United States, the diagnosis of cutaneous diphtheria first (18) and then leishmaniasis must be considered in the consideration of ulcerative cutaneous lesions. For the other forms of cutaneous leishmaniasis the differential diagnosis indeed must consider many of the infectious granulomata of the tropics. This list includes, of course, the mycoses, especially sporotrichosis, blastomycosis, chromoblastomycosis, paracoccidiosis, yaws, syphilis, granuloma inguinale (extra-genital), rhinoscleroma, tuberculosis (lupus vulgaris), leprosy, stasis syndromes, etc (figures 7, 8, 9, 10). To complicate the picture, some of these, especially the deep mycoses and syphilis, may coexist with cutaneous and mucosal leishmaniasis. More detailed cultural studies of cutaneous and mucosal leishmaniasis will serve to reveal the extent of secondary invaders. Especially with the non-ulcerative types of cutaneous and mucosal leishmaniasis, the inexperienced dermatologist, even in endemic zones, must rely on laboratory measures. The ideal state is to find the organisms (figure 11). This is often very difficult in the chronic lesion, or in the lesions overwhelmed by secondary infection, and in the heavily scarred lesion. In most instances, the instructions given in standard texts for the pipette aspiration of the periphery of the lesion are preferred to careless or casual scrapings of the lesion. In order to obtain fluid for smears, Weiss, Herrer and O'Hara have tried the clamp squeezing technic, such as is used to obtain spirochaetes and also leprosy bacilli in fluid from skin. These workers were not successful. Benedek (19) has claimed the demonstration of leishmania, repeatedly, in the contents of cantharis blisters from a patient in the United States. This patient had an extensive ulcerative lesion, supposedly leishmaniotic, of the face associated with osteomyelitic foci (of unreported bacterial nature) of the right humerus, right clavicle, left ulna and the right astragalus. This technic has not been confirmed. The organisms isolated were

reported to cause the same disease in guinea pigs. An abundance of parasites on direct examination of the lesion of American cutaneous leishmaniasis is an uncommon occurrence. Great caution must be exercised by the inexperienced



FIG. 7. SPIROCHETAL LESIONS SIMULATING AMERICAN CUTANEOUS LEISHMANIASIS

FIG. 7A. Late syphilis with gummatous ulcer of the cheek.

FIG. 7B. Late syphilis of the skin.

FIG. 7C. Yaws.

FIG. 7D. Gangosa.

observer, for without special stains such as Giemsa, yeast forms may be misdiagnosed as round *Leishmania* forms. Those who have had most experience and are careful in their technic of collection of material for culture, believe that cultural methods with the N. N. N. media or its modifications are valuable.

Dostrovsky and Sagher believe the culture method has definite practical diagnostic significance. In field work, it is not always possible to take cultures. Secondary infection appears to be the difficulty in securing adequate cultures of *Leishmania*. In the hands also of experienced workers, egg embryo cultures are also of value. Attempts at diagnosis of type of *Leishmania* concerned with

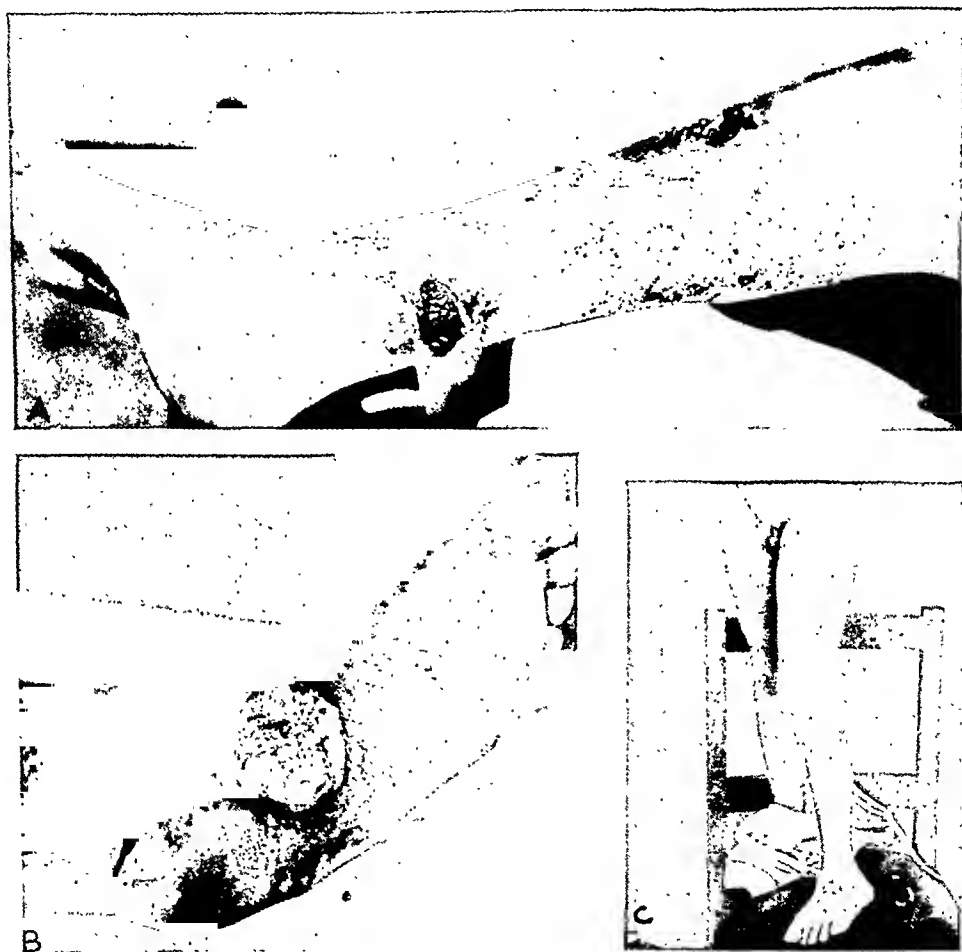


FIG. 8. SOME ULCERATIVE LESIONS OF THE EXTREMITIES TO BE CONSIDERED IN THE DIFFERENTIAL DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS

FIG. 8A. Chronic pyogenic forms.

FIG. 8B. Tumor with ulcer, in this instance a melanoma amelanotic.

FIG. 8C. Tropical ulcer syndrome with adenitis.

agglutinin reactions and the like should be left to experienced parasitologists. The leishmanin skin test (Montenegro test) is reported to be of great value in cutaneous and mucosal forms. This vaccine is prepared with washed flagellates in physiological saline with 0.4 per cent phenol. The Doubovsky vaccine (24) used for studies in cutaneous leishmaniasis in the Soviet Union is prepared from cultures in a milk medium. For estimation of the allergic response, Sagher has used graded intracutaneous doses of leishmania vaccine. The leishmanin test

should be used more extensively in critical field work in edemic zones of American cutaneous leishmaniasis. As yet, xeno-diagnosis with *Phlebotomus*, *Triatomata* or even *Cimer* is not of practical value. In my brief experience, biopsy studies of the well developed lesion with hematoxylin-eosin and trichrome stains has not



FIG. 9 SOME DEEP MYCOSES TO BE CONSIDERED IN THE DIFFERENTIAL DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS

FIG. 9A. Blastomycosis.

FIG. 9B. Chromoblastomycosis.

FIG. 9C. Sporotrichosis with deep gummatous-like nodules.

FIG. 9D. Sporotrichosis with deep lymphangitic type nodules.

FIG. 9E. Sporotrichosis with superficial lymphangitic nodules.

been of any practical value in differential diagnosis procedures. Non-specific inflammatory and tuberculoid reactions were observed (figure 12). Intra and extra cellular organisms are reported in biopsies especially of early lesions. In a review of the autochthonous case of alleged cutaneous leishmaniasis in Texas, reported recently by Stewart and Pilcher (20) (culture negative) Wenyon (21) indicated that "it is unfortunate that leishmania were not found in Giemsa



FIG. 10. SOME CHRONIC FACIAL LESIONS TO BE CONSIDERED IN THE DIFFERENTIAL
 DIAGNOSIS OF AMERICAN CUTANEOUS LEISHMANIASIS

- FIG. 10A. Lupus vulgaris.
- FIG. 10B. Sarcoidosis with nasal, lip, and mucosal involvement.
- FIG. 10C. Ecthymatous syphilides.
- FIG. 10D. Extra-genital granuloma inguinale with facial and mucosal involvement.
- FIG. 10E. Basal cell malignancy.
- FIG. 10F. Facial pyoderma following pyodermic myiasis.

stained smears of any of the lesions, for identification of these parasites in sections is always open to question unless made by an observer who has had extensive experience with these particular organisms. The microphotograph illustrating the paper is a good one but although suggestive of leishmania it is not absolutely convincing." Igochine and Tscherniak are reported by Hoare (24) to advocate capillaroscopy as a "subsidiary method" for the diagnosis of oriental sore. One case of oriental sore was studied with special skin microscopy technics by the author (25) but no definite diagnostic features could be made out. A monocytosis of the circulating blood is reported in cutaneous leishmaniasis. No detailed reports relative to blood protein studies were found for American cutaneous leishmaniasis. Blood cultures by Mazzo and Niño in 392 cases in Peru were negative.

As would be expected, there has not been too much interest in the general aspects of the patient with primary cutaneous leishmaniasis. His associated visceral involvements frequently are found or assumed to be due to the other common visceral diseases of the tropics. Although it has been stated that American cutaneous leishmaniasis may involve the lymphatic system, lymph glands adjacent to cutaneous lesions show no significant enlargements unless there are severe secondary infections associated. No gland punctures examinations for leishmania were done. Weiss and O'Hara have also indicated no marked lymph glandular enlargements in cutaneous leishmaniasis in Peru. Any studies concerned with the background of cutaneous leishmaniasis must include of course, complete physical examinations and laboratory studies of the patients.

What are the relationships between the variants of the cutaneous reactions of American leishmaniasis, between American and Old World leishmaniasis, and between cutaneous and visceral leishmaniasis? Such widely diverse reactions can truly be considered together. American cutaneous leishmaniasis is found from Mexico to North Argentina, with Peru and Brazil the heaviest centers of infection. American visceral leishmaniasis is found, for the most part, in North and East Brazil, the Matto Grosso region, the Chaco region of Argentina and Northern Bolivia, and Yungas in Bolivia. O'Hara claims that no kala-azar as yet has been found in Peru. As with so many other tropical diseases in the Americas, detailed studies will certainly enlarge the field of both these forms of leishmaniasis. For the most part, save perhaps in Mexico and Brazil (?), American cutaneous leishmaniasis is chiefly a disease of the young, although all ages, all races and all occupations may be included. As with other infections in infancy, it is not found before six months of age. Even the foreigner working in the endemic areas of the Americas may get leishmaniasis. As a rule, the manifestations are lesser in severity in the young. Observers in Peru claim that the severity varies even from area to area in the endemic zones, and also that strangers in an area acquire a more severe form of leishmaniasis than the native inhabitants of an area. The cutaneous lesions in Mexico appeared more benign than even those in Costa Rica, and the lesions in the adult in Peru appeared to be much more severe. Brazil and Paraguay also are reported to show the severe forms. However, clinical forms follow the basic theme of development of

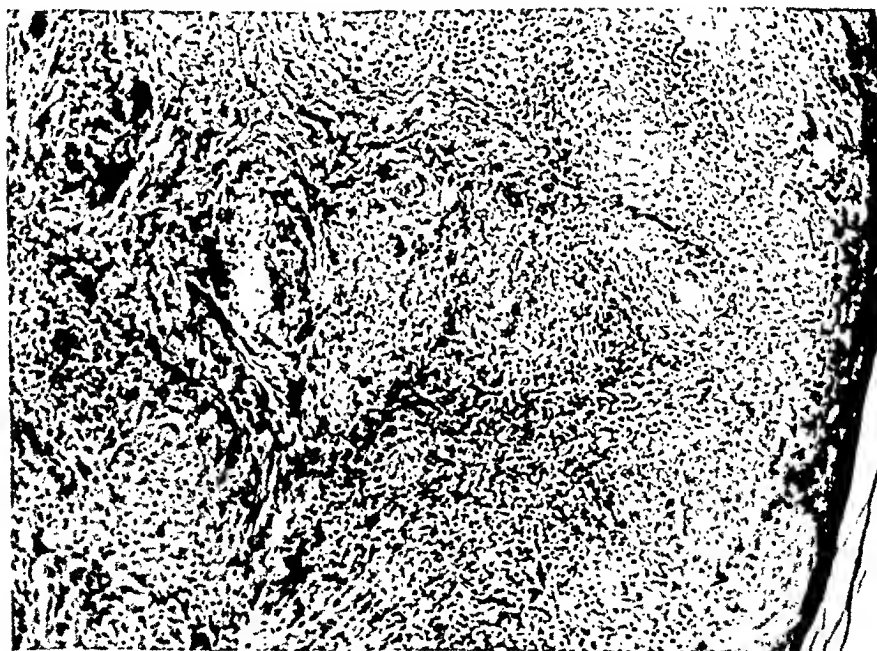
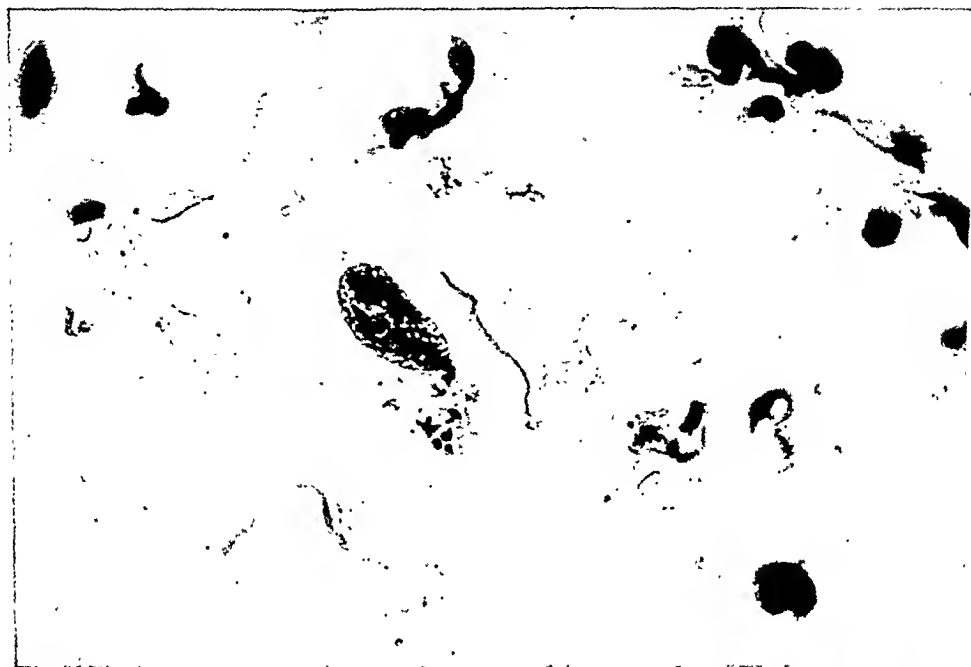


FIG. 11 (top). SMEAR FOR LEISHMANIA, PT. H.-HOSPITAL LOAYZA, LIMA PERU
(WEISS-O'HARA EBELL)
May Grünwald-Giemsa $\times 480$

FIG. 12 (bottom). BIOPSY OF A SERPIGINOUS LEISHMANIA ULCER OF THE FOREARM
(FIG. 1D) SHOWING NON-SPECIFIC TUBERCULOÏD REACTION
hematoxylin-eosin $\times 120$

cutaneous leishmaniasis. The metastatic mucosal lesions appear chiefly in the selva region "en toda la selva Tropical Amazonica," and also chiefly in adults. Even in the selva, the incidence of this mucosal form of leishmaniasis varies according to the resistance of the racial group involved. Some indigenous tribes are apparently very resistant to leishmaniasis in their region. It appears then, that although there are such factors as organism virulence, vector relationships, and tropism (cutaneous, mucosal, visceral), and local factors such as treatment, secondary coccal and mycotic infections, etc., and systemic factors such as avitaminosis (Peña Chavarría), in spite of all these, the characteristic of immunity (acquired and inherited ?) is important. Such speculation could be proved, especially in Peru and Brazil, by detailed statistical analyses, cutaneous testing, inoculation studies and the like. Analogies to Old World leishmaniasis are frequent especially in all discussions relative to variations of clinical pictures. It is well to note that the lesions in the American Forces in the Middle East were for the most part not extensive, chiefly because of early diagnosis and early and adequate therapy (and adequate diet ?). Is there an ancient heritage of cutaneous leishmaniasis in the Americas? One must be careful of interpreting the marvelous realistic studies in ancient ceramic pathology of the Peruvian Chimu and Muchick periods, because even inspection diagnosis of an excellent colored clinical photograph is not without danger. Such deformities in the *huacos*, even from *uta* regions, may represent, according to Tello (22) and Lastres (23), punitive mutilations and other infections as well as leishmaniasis. Nevertheless, the *uta* type is suspected of being very ancient in America. *Uta*, according to Weiss, is a Quechua Indian word meaning *gnaw* (roer, carcomer). It is striking to go into these villages now and see almost universal cutaneous involvement. There can be no doubt about the clinical similarity between American and Old World cutaneous leishmaniasis. As noted previously, the Peruvian, José Julian Bravo, as early as 1852 recognized the *uta* form in Peru as similar in character to the oriental sore. Arce claimed that the similarity was based on common features of endemicity in limited areas, onset preferably in children before four years of age, a benign evolution and rarely relapse. Weiss does not subscribe to the last two reasons because of the lack of critical statistical data now available for the Americas. Escomel believed the Peruvian cutaneous leishmaniasis, in regard to clinical severity, occupied a position midway between American cutaneous leishmaniasis and Old World leishmaniasis. The finding of leishmania organisms in 1909 in Brazil by Lindenberg, Carini and Paranhos showed the general organism relationships. Even the clinical variants in the picture of American cutaneous leishmaniasis, including leishmaniasis multilans auriculi (Gill), and nodular lymphangitis types, are reported in oriental sore. Moreover, the Russian classification (24) of *dry* and *moist* type of cutaneous leishmaniasis can also be applied in a general fashion to American cutaneous leishmaniasis. Peña Chavarría would consider the ulcerative lesions as the moist type, and the nodular and verrucous as the dry types. However, these two types appear to have sharp differences according to Russian workers, and such pure forms with such specific definite properties can not be seen too readily,

at least in the extensive *uta* endemic areas of Peru. Yet, *dry* and *wet* forms are certainly found. Hoare (24) indicates that detailed work in Middle Asia has a direct bearing on the position in other countries, since *moist* and *dry* forms can be seen. The immunity studies of Kojenikov (26) are interesting, in that in regard to *moist* and *dry* forms, he suggests "cases of reinfection are especially liable to occur on transfer from one endemic area to another." He adds, "it is concluded that cross-immunity between the two types of oriental sore is absent or only slightly developed and that the course of infection with a heterologous type is milder than in the primary disease." There is no evidence in the Americas as yet, that there be two distinct immunological types of cutaneous leishmaniasis. Again, the endemic areas here would provide excellent opportunities for pioneer work in immunobiology of leishmaniasis. As yet only in Brazil have such studies been initiated.

The real clinical distinction of the American form is the metastatic mucosal lesion first presented by Carini in 1911, and Splendore in 1913. What is the origin of metastatic mucosal leishmaniasis? No one knows. *Espundia*, according to Weiss, is a Spanish word used in veterinary medicine, indicative of tumors which ulcerate. Is it a development of the cutaneous type, modified by the general effect of the selva areas? The depiction of pathology in ancient ceramics was confined chiefly to the coastal and adjacent areas. The mucosal form of leishmaniasis was described early during the Spanish domination. According to Palma, in 1586 Fray Rodrigo de Loayza told of the resistance of the Indians to a disease affecting the nose of those entering certain regions of the Andes (cordillera oriental?). Even today it has been noted that leishmaniasis is rare among the "barbaric tribes of the selva." Perhaps there is, then, a certain racial immunity. There is no evidence at present regarding cross-immunity studies between the *uta* and the *espundia* regions. Such metastatic mucosal lesions are not present in Old World cutaneous leishmaniasis. However, the efforts of Kirk (27, 28) to integrate the two forms of cutaneous leishmaniasis with visceral leishmaniasis are of interest, "actually there is no hard and fast line of demarcation between these three types." He explains this on the basis of parasite strain behavior through virulence and adaptation changes. He describes, in kala-azar in the Anglo-Egyptian Sudan, metastatic mucosal leishmaniasis associated with the visceral leishmaniasis and reports an experimental mucosal infection by the inoculation of a monkey series with Sudan Kala-azar. In regard to post visceral dermal leishmaniasis, he believes the cutaneous lesion may be ulcerative in untreated or non-ulcerative following the successful treatment of the visceral infection. It has been assumed that possibly because of different vectors, kala-azar in the Sudan is not identical with the Indian or Mediterranean varieties. *Leishmania* have been reported by Forkner and Zia (9) in China on the nasal mucosa and tonsils of kala-azar. In the Americas, it is significant when both visceral and cutaneous leishmaniasis are reported in villages which are relatively isolated. Such is the report from Colombia by Gast and Rengifo (29) in 1944. It is hoped that with increasing knowledge of the mechanism of leishmania infection in man (and in the animal reservoirs) the

efforts of Kirk (27, 28) to establish pathogenetic relationships will be established. Detailed examinations of the viscera, commonly assumed to be an associated malarial involvement, and detailed laboratory studies of the patients with American cutaneous leishmaniasis should be done.

Students of immunobiology (30), then, will find a fruitful field of research in cutaneous leishmaniasis. Many of these characteristics have been considered, such as the antiquity of the disease as related to possible racial immunities, the regional variations of the clinical picture of the disease in the Americas, the age incidence in endemic areas of cutaneous and mucosal leishmaniasis. Other important procedures in studies in immunobiology are the inoculation studies of primary inoculation, reinfection and superinfection attempts, post-lesion excision inoculation, etc., and especially in the Old World leishmaniasis, vaccination work, leishmanin cutaneous testing, and the investigation of the specificity of parasite strains.

Salts of antimony continue to be the systemic treatment of choice for cutaneous leishmaniasis. Whether antimony tartrate or trivalent organic antimony such as fuadin (sodium antimony biscatechol disulfonate of sodium) or pentavalent antimony such as neostam (nitrogen glucoside of sodium p. amino phenylstibonate) is used depends upon chiefly the feelings of the observer. Occasionally intra-muscular injections may be preferred to intravenous injections. Peña Chavarría has used oral tartar emetic especially in children. The badly infected, the chronic scarred but active lesion, and the mucosa, especially the metastatic form, do not appear to respond well, and at times not at all, to antimony therapy. "La uta es mucho mas docil al tratamiento que la Espundia" (Weiss) (1). Occasionally, the clinical procedure which we have used in the chronic granuloma inguinale case may be employed; this consists in changing to another antimony compound. The recent work of Braun, Lusky and Calvery (31) in the use of BAL (2-3 dimercatopropanol) in antimony poisoning suggests that this compound may be used in severe toxic reactions in man from antimony compounds. Weiss has mentioned a Herxheimer reaction from antimony in the treatment of a tracheo-bronchial leishmaniasis. Anthiomaline and many of the newer antimony compounds used in kala-azar have also been tried in cutaneous leishmaniasis. It is very important in the adult especially that injections be given "long enough" to avoid relapses. The attempted correction of any associated systemic condition such as avitaminosis, intestinal parasitism, tuberculosis, malaria, etc. of course is indicated. The value of polyvitamins, added just as a general measure, is not known. Peña Chavarría believes these and adequate dietary measures to be important. As mentioned previously, the influence of the systemic condition on the extent of the lesion is suspected but not evaluated critically. Some other agents which have been used are tissue extracts and even subcutaneous injections of preserved aloe leaves as recommended by Filatov (32) in cutaneous leishmaniasis in Russia. Vaccine therapy has been recommended also as a therapeutic aid. Arsenicals have also been used, especially in cases where syphilis also may be active or suspected in the clinical picture. The pentavalent arsenical, melarsen, has also been tried. Iodobis-

mutate of quinine is mentioned by Gutiérrez. There are no reports as yet of systemic therapy with streptomycine.

A variety of local therapies have also been tried. The conventional wet dressing types, such as diluted Burow's with bichloride, D'Alibour, red cell pastes, etc. have been suggested. In addition, the following have been used, sulfonamides and penicillin for the local infection, local injections of berberine sulfate 2 per cent neostam and atehrin, local cantherizing with acids ethyl chloride and carbon dioxide, etc. According to Romero (38) five per cent sulfathiazole ointment is of help when there is not much cellulitis about the ulcer. Grenz rays have been used in Palestine for Old World leishmaniasis and x-ray therapy recommended in Peru especially for the mucosal case and for elephantiastic types. Electro-surgery may also be employed. It appears that plastic surgery has a role for these extensive facial deformities from old or neglected or badly infected facial leishmaniasis. For many reasons, this form of surgery has not been an active or practical interest at present. One must be certain that the tissue and especially the fibrotic margins are free of leishmaniotic foci before plastic surgery is done.

The prevention of cutaneous leishmaniasis in the endemic areas appears to be a well nigh hopeless affair. It is likely the incidence could be reduced considerably by definite knowledge of vectors, animal reservoirs and transmission, etc., prophylactic vaccine therapy, educational programs to secure early and adequate, and not folk lore therapy, especially in the young, the insistence on maintained therapy and observation for the extensive case in the adult. Spontaneous cure does occur, of course, but sometimes not until extensive tissue damage has been done. The problems of control in the selva are even greater. One has only to read Kuczynski-Godard (14) to appreciate some of the difficulties of the control program here. If the foreigner in the endemic area wishes some simple practical advice, the use of modern repellents, insecticides, adequate fine mesh screening, and the other measures for the maintenance of careful hygiene of the skin in the tropics, and early attention to indolent lesions on exposed parts of the body may be suggested. With regard to prophylactic vaccination for American leishmaniasis, it is not possible at present, to give any definite recommendations, at least for Mexico, Central America and Peru. The detailed studies have been done by Pessoa and Pestana of Brazil. It is not known as yet whether there are type specificity strains for the different endemic areas of leishmaniasis, and whether therefore, a so-called "polyvalent vaccine" should be used.

CONCLUSIONS

Primary American cutaneous leishmaniasis follows the basic clinical pattern of primary cutaneous leishmaniasis elsewhere, with the inoculation, papule, ulcer, and scar phases. Variants of this pattern exist, and some regions show more frequent and characteristic clinical forms. Metastatic mucosal leishmaniasis is the outstanding characteristic of American cutaneous leishmaniasis. There are many unknown important features of primary cutaneous leishmaniasis,

including especially epidemiology and immunobiology. Further investigative studies with cutaneous leishmaniasis, and even with visceral leishmaniasis may show reasons for the clinical type variations not only in the Americas, but also with respect to cutaneous leishmaniasis of the East and that of Middle Asia, and may help to support Kirk's attempts to establish connections between the various types of cutaneous and visceral leishmaniasis.

The author is grateful to Professor Pedro Weiss and Doctor Herbert O'Hara Ebell of Lima, Peru, and Dr. Antonio Peña Chavarría of San José, Costa Rica, for their help in the preparation of this report.

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SOME CHARACTERISTICS OF FOREIGN VIVAX MALARIA INDUCED IN NEUROSYPHILITIC PATIENTS^{1, 2}

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Received for publication March 26, 1947

During the program of studying foreign malaria imported by returning service men, it has been shown that these strains are infective to and can be transmitted by our native malaria vectors (7, 9, and 11). Neurosyphilitic patients were inoculated therapeutically and from these patients certain data were obtained bearing upon the host-parasite relationship. These observations form the basis of the present report.

METHODS

Practically all patients treated were service men. While under malaria-therapy, oral temperatures were taken every 4 hours except during fevers when temperatures were taken hourly or even more frequently. During the fever, patients received the usual symptomatic care accorded those undergoing malaria-therapy.

Most of the infections were transmitted by bites of infected mosquitoes which were mainly *Anopheles quadrimaculatus*; some infections were induced by blood transfer. Blood smears were taken at least once daily. Density counts of malaria parasites were made by the Earle-Percz (2) method with about 0.1 cmm. of blood examined as a minimum.

Although a total of 27 strains was employed, the principal strains of malaria used were four from the Pacific area and one from the Mediterranean area. One of the Pacific strains used (v-1027-NG) apparently originated in New Guinea and has been designated the "Chesson" strain (3). This strain has been used extensively in the investigation of new anti-malarial drugs.

OBSERVATIONS

Prepatent and Incubation Periods (Mosquito Induced Malaria). Usually from 5 to 10 infected mosquitoes bit the recipient patient, but in a few cases the number varied from 1 to 22.

Data were available in 123 cases for both the prepatent and incubation

¹ This is the sixth in a series of reports on imported malarias. The complete title of this report reads, "Studies on Imported Malarias. 6. Some Characteristics of Foreign vivax Malarias Induced in Neurosyphilitic Patients".

² Contribution from the Imported Malaria Studies program of the Office of Malaria Investigations, National Institute of Health, and the Office of Malaria Control in War Areas, United States Public Health Service, Columbia, S. C.

The Harmon General Hospital and the South Carolina State Hospital furnished laboratory space and made possible the securing of the information upon the induced infections. To the staffs of these hospitals we express our appreciation as well as to the Office of the Surgeon General, United States Army, whose active interest made the program possible.

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periods. These data are given in table 1. It is apparent from the table that the Mediterranean strains of *P. vivax* gave rise to infections in which the developmental period was shorter than in strains of malaras from the Pacific or Burma area. To test this further, the strains were compared statistically, the most frequently used strains from the Pacific and Mediterranean areas being chosen

TABLE 1

Prepatent and Incubation Periods of Foreign P. vivax Induced in Neurosyphilitic Patients
Transmission by *A. quadrimaculatus*. Only first inoculations included. Malaras arranged by areas of origin

AREA OF ORIGIN	WHITE PATIENTS				NEGRO PATIENTS				TOTAL PATIENTS			
	Number of strains	Number of patients	Pre-patent period, av. days	Incubation period, av. days	Number of strains	Number of patients	Pre-patent period, av. days	Incubation period, av. days	Number of strains	Number of patients	Pre-patent period, av. days	Incubation period, av. days
Pacific.....	18	86	13.1	14.4	3	3	15.3	16.0	18	89	13.2	14.4
Mediterranean.....	8	30	12.1	13.7	2	3	14.3	18.3	9	33	12.3	14.1
Burma.....	1	1	15.0	17.0					1	1	15.0	17.0
Total.....	27	117	12.8 ±.17*	14.2 ±.19*	5	6	14.8	17.2	28	123	12.9	14.3

* A test of significance applied to these means shows a difference of 5.29 standard errors which indicates that the difference between the prepatent and incubation period is real.

TABLE 2

A Comparison of a Pacific and a Mediterranean Strain of Malaria for Prepatent and Incubation Periods

White patients only

STRAINS	PREPATENT PERIODS		INCUBATION PERIODS	
	1027-NG	1031-Si	1027-NG	1031-Si
Cases.....	36	21	36	21
Means (days).....	12.8100	11.8571	13.7778	13.0476
S.D. individual length.....	1.6808	1.3553	1.8573	1.3619
S.D. mean length.....	0.2801	0.2985	0.3096	0.2999
Standard error.....	2.4991		2.3585	

NG—New Guinea; Si—Sicily. Strain 1027-NG is also known as the "Chesson" strain.

for the comparison. The results, which are shown in table 2, indicate that infections caused by the Mediterranean strain had significantly shorter prepatent and incubation periods than did infections caused by the Pacific strain.

Parasite-Fever Threshold. Daily quantitative counts were made on 35 patients who had been infected by mosquitoes to determine the parasites per cmm. on the first day of fever (100 F. or over). Thirty of these patients (17 with a

Pacific and 13 with a Mediterranean strain) had a primary attack of over 9 days and were considered as having no immunity. The 17 patients with a Pacific strain (1027-NG) averaged 21 parasites per cmm. on the first day of fever; the number of parasites ranged from 3 to 60 per cmm. (fig. 1). The 13 patients with a Mediterranean strain (1031-Si) averaged 45 parasites per cmm. on the first day of fever with the number ranging from 1 to 90 per cmm. Five other patients with a Pacific strain (1027-NG) had symptoms lasting 8 days or

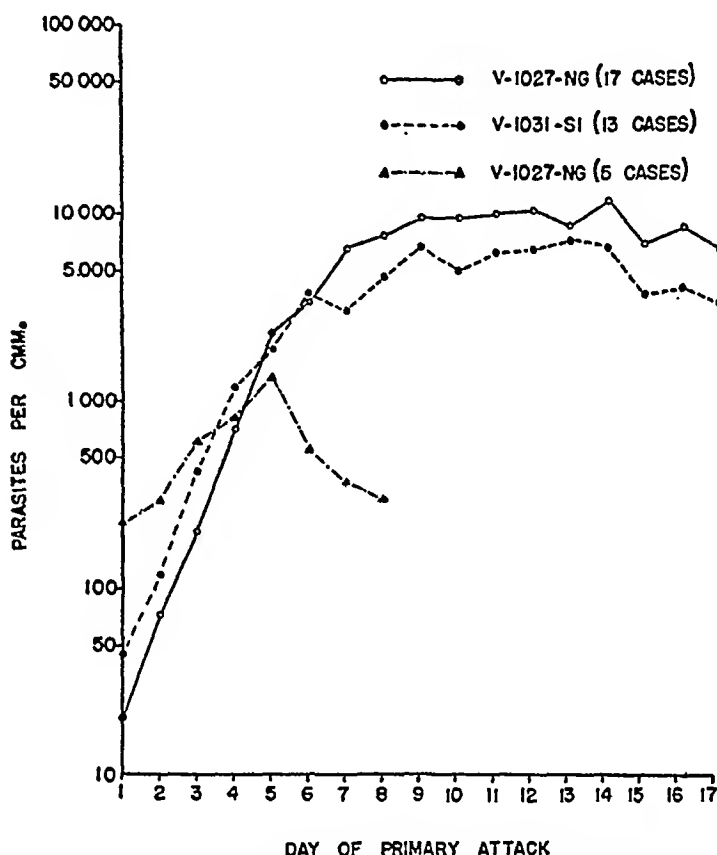


FIG. 1. RELATIONSHIP OF PARASITEMIA TO DAY OF PRIMARY ATTACK IN FOREIGN MALARIAS TRANSMITTED BY MOSQUITOES.

Five cases of v-1027-NG exhibiting a short primary attack apparently had some immunity. The other 30 cases apparently experienced a primary attack uncomplicated by immunity.

less. The average number of parasites per cmm. the first day of fever was 229, the range being from 5 to 882 per cmm. However, the parasitemia did not reach high levels, and the patients soon maintained normal temperatures. The relatively high parasite count during the first fever, the short duration of symptoms, and the relative low overall parasitemia indicate an immunity in these 5 patients.

Relationship of Parasitemia to the Symptoms. For the 35 patients just mentioned, the relationship of the average parasitemia to the day of primary attack

is shown in figure 1. Other data were available on the 35 patients and an additional 30, all of whom had received foreign strains of malaria by mosquito bites (table 3).

The maximum temperature is defined as the highest temperature recorded during the course of the fevers. If more than one maximum temperature occurred, the first was used in these tabulations. It is seen from table 3 that the average for the 65 cases was 106.0° F. The maximum temperature usually preceded the maximum parasitemia by several days, both tending to occur, however, in the second week of the primary attack. The maximum temperature sometimes occurred when a relatively small number of parasites were present,

TABLE 3

Relationship of Parasitemia to Fevers in Neurosyphilitic Patients with Foreign vivax Malarias

STRAIN	NUMBER OF PATIENTS	FIRST MAXIMUM TEMPERATURE				MAXIMUM PARASITEMIA			
		°F.		Parasites/cmm.*		Parasites/cmm.*		°F.	
		Range†	Average	Range	Average	Range	Average	Range†	Average
<i>Mosquito inoculated</i>									
1005-G	5	5.4-7.4	106.4	2.0-13.3	5.7	6.8-13.3	10.5	4.0-5.8	105.1
1019-G	3	5.2-5.8	105.5	0.1- 2.8	1.4	1.5- 3.1	2.3	5.0-5.2	105.1
1027-NG	34	4.0-7.0	105.9	0.3-44.2	8.1	1.0-44.2	15.6	2.0-6.4	105.0
1031-Si	16	5.2-6.8	106.3	0.2-13.1	3.8	3.2-35.4	12.8	3.0-6.4	104.7
1032-NG	7	5.8-6.2	106.0	0.5- 9.0	3.6	5.5-43.2	14.5	5.0-6.2	105.4
Total	65	4.0-7.4	106.0	0.1-44.2	6.2	0.8-44.2	13.9	2.0-6.4	105.0
<i>Blood inoculated</i>									
1027-NG	10	5.6-6.4	105.7	1.2-42.8	10.2	6.5-55.3	25.3	4.0-6.2	105.4

* In thousands.

† Degrees above 100 F.

e.g., 100 per cmm. Parasitemias many times as great occurring later in the primary attack often failed to elicit as high a fever response.

The maximum parasitemias averaged 13,917 parasites per cmm., as compared to an average of 6,207 per cmm. which accompanied the maximum temperatures.

Thus, it is apparent that the fever response was not in direct proportion to the number of parasites present. The lesser febrile response to a higher parasite density in the latter part of the disease probably signifies a developing immunity against the effects of the parasites.

The highest parasitemias seen were 44,200 per cmm. for the Pacific strain (1027-NG) and 35,400 per cmm. for the Mediterranean strain (1031-Si).

From tables 3 and 4 and figure 1, it appears that the Mediterranean strain (1031-Si) did not produce an average parasitemia as high as the Pacific strain but that the fever response was about the same.

Of the 34 cases infected with strain 1027-NG, a comparison of the parasitemias according to whether the fevers occurred daily or every other day is as follows:

STRAIN 1027-NG	FEVERS	
	Mainly quotidian (27)	Mainly tertian (7)
Parasites per cmm. at first maximum temperature..	7,100	12,200
Parasites per cmm. at maximum parasitemia.....	14,700	18,900

One might have expected that patients with quotidian fevers, indicating the presence of two broods of parasites, would have higher parasitemias than those with tertian fevers indicating only one brood of parasites. Such, however, was not the case.

There were data available on 10 patients who received strain 1027-NG by blood transfer, and these are also shown in table 3. Five of these received thio-

TABLE 4
Average of Fever Peaks in 5 Strains of Induced Foreign Malarias

STRAIN	TOTAL PATIENTS	TOTAL PAROXYSMS	AVERAGE FEVER PEAKS
			°F.
1005-G	5	58	104.7
1019-G	3	38	104.4
1027-NG	37	432	104.4
1027-NG*	11	120	104.7
1031-Si	16	210	104.6
1032-NG	7	76	104.8
Average.....	79	934	104.5

* Blood induced. Remainder induced by mosquito bites. NG—New Guinea; G—Guadalcanal; Si—Sicily. Strain 1027-NG also known as the "Chesson strain".

bismol early in the infection which may have influenced the time of appearance of the maximum temperatures and parasitemias. In general, the blood induced infections had higher parasitemias than the sporozoite induced infections and (although not shown in table 3) the maximum parasitemias and maximum temperatures tended to occur more nearly together than in the sporozoite induced cases. This was true whether or not the blood induced cases had received thio-bismol.

The Height of the Fevers. The highest temperature reading in each paroxysm above 100° F. was called the "fever peak", and these were tabulated for 934 paroxysms occurring in 79 patients. These data are shown in table 4. The fever peaks averaged 104.5° F.

The height of the fevers in relationship to duration of the primary attack is shown in figure 2 where the average fever peaks for both blood induced and mosquito induced infections are shown. The strain of malaria was 1027-NG (Chesson).

The fever peaks gradually increased during the first paroxysms. The maximum was reached usually during the second week of symptoms. This was true of all 5 strains on which data were available.

In the blood induced infections the fever peaks rose faster and maintained a higher level generally than in mosquito induced infections. As shown above, this was also true of the parasitemia in blood induced infections.

Periodicity of Fevers. To calculate the periodicity of the fevers, the peaks of the fever were used as reference points; the intervals between these peaks are designated as "paroxysmal intervals".

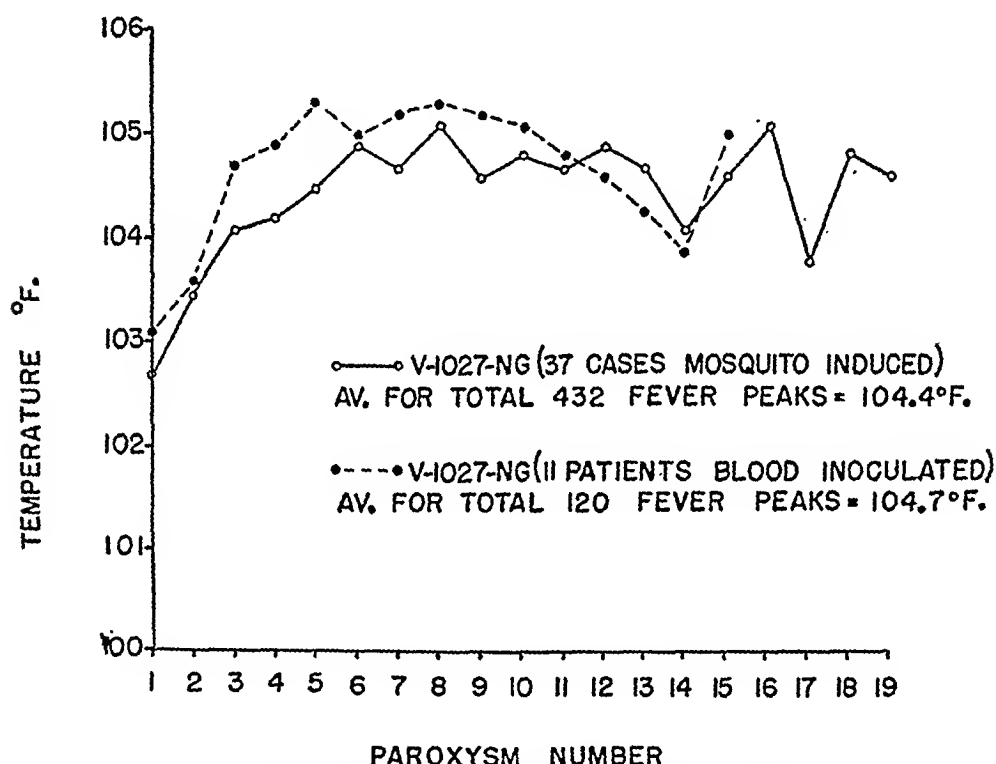


FIG. 2. THE RELATIONSHIP OF THE FEVER PEAKS TO THE NUMBER OF PAROXYSMS EXPERIENCED IN THE PRIMARY ATTACK.

The New Guinea strain (Chesson) of *P. vivax* was induced either by blood or mosquito inoculations.

As the temperature readings were taken every 4 hours and hourly when over 100° F., the peaks were evident. When the fever remained at peak for more than one reading, the first reading was used. It has been shown (8) that the fever peaks have a definite time relationship to the segmentation phases of the parasites, so that the length of time between fever peaks can be taken as the length of the asexual cycle of the parasites.

These intervals were counted only after the fevers had settled down to a tertian rhythm, either naturally or following the use of thio-bismol (8). The hours between fever peaks were measured and tabulated as whole units, being

figured to the nearest hour. The total fever intervals studied were 260, and the results are shown in table 5.

None of the strains showed a 48-hour periodicity; all were of a shorter duration. The average for all strains was 44.5 hours with the range in the 5 strains averaging between 43.6 and 45.1 hours.

TABLE 5
Periodicities of Tertian Fevers of Foreign P. vivax Malarias

	PACIFIC STRAINS				PACIFIC STRAINS TOTAL	MEDITERRANEAN STRAIN 1031-Si	ALL STRAINS TOTAL
	1005-G	1019-G	1027-NG	1032-NG			
No. of patients.....	3	2	18	7	30	7	37
No. of paroxysmal intervals.....	19	19	133*	47	218	42	260
Mean length of paroxysmal intervals (hours).....	45.0	43.6	44.3	44.8	44.4	45.1	44.5
S.D. mean (hours).....	0.35	0.44	0.15	0.28	0.12	0.35	0.12

* Includes 6S intervals from blood induced cases. Remainder from mosquito induced infections.

TABLE 6
Percentage of Paroxysms Accompanied by Chills

Arranged according to stage of disease. Only patients showing a course of more than 5 paroxysms were included.

STRAIN NUMBER	PERCENTAGE OF PAROXYSMS ACCOMPANIED BY CHILLS				TOTAL PAROXYSMS	
	Paroxysm number				Number	Per cent with chills
	1-5	6-10	11-15	16-20		
1005-G	64.2	96.0	88.9		59	81.4
1019-G	53.3	66.7	83.3		36	63.9
1027-NG	52.7	80.3	94.4	89.5	420	72.9
1031-Si	58.2	88.5	100.0	100.0	144	79.9
1032-NG	45.7	75.0	70.0		77	61.0
Total.....	53.9	81.9	92.9	93.3	736	73.2
Total paroxysms.....	295	271	140	30	736	

Relationship of Chills to Paroxysms. The association of chills with the fevers was examined in 736 paroxysms. The patient was designated as having a chill if he complained of being cold, as well as when the overt chilling process was observed. The data are given in table 6.

From these data it is clearly shown that proportionately fewer chills accompanied the first five paroxysms than the paroxysms after the fifth.

Of the 420 paroxysms shown for 1027-NG, 325 were from blood induced

infections, and the rest were mosquito induced. Using the fourfold table, the difference between these two methods of the association of chills and fevers was shown to be 1.8 Standard Errors which is not taken to be significant. Of the total 736 paroxysms, 416 were from blood induced infections. The difference between blood and mosquito induced methods for the total number was also insignificant (0.1 Standard Error).

As the maximum parasitemias usually occurred after the fifth paroxysm, this suggests that the rigors might have some relationship to the number of parasites present.

Type of Fever at Onset. The type of fever at onset in 58 cases induced by mosquito bites follows:

SEQUENCE OF FEVERS	NO. OF CASES	TOTAL	PER CENT OF TOTAL CASES
Remittent followed by:		24	42
quotidian solely.....	14		
tertian solely.....	6		
quotidian, then tertian.....	3		
tertian, then quotidian.....	1		
Quotidian followed by:		29	50
quotidian solely.....	18		
tertian solely.....	9		
tertian, then quotidian.....	2		
Tertian followed by:		5	8
tertian solely.....	4		
quotidian solely.....	1		

One-half of the cases started as quotidian, 42 per cent as remittent, and only 8 per cent as tertian. Of those changing eventually to tertian, regardless of onset type, there were 22 (38 per cent) and those changing to quotidian eventually were 36 (62 per cent). Most of those which started either as quotidian or tertian continued as such. Only a few starting as remittent fever remained so long enough (3 to 4 days) to necessitate using thio-bismol (sodium bismuth thioglycollate) to convert them to tertian periodicity. These were not included in the above table.

Of 8 blood inoculated cases studied, none started as remittent, 2 started and remained tertian, 2 started quotidian and changed to tertian, one showed a quotidian-tertian-quotidian development, 2 started and remained quotidian, and one started tertian and changed to quotidian.

The 24 remittent fevers were distributed according to duration as follows: 2 days, 9; 3 days, 9; 4 days, 6.

Use of Sodium Bismuth Thioglycollate to Convert Paroxysms to Tertian Periodicity. Sodium bismuth thioglycollate (thio-bismol) was given when one brood of parasites was half-grown to convert remittent fevers to a tertian periodicity. It was given also to 12 cases of quotidian paroxysms, and 11 were changed to

tertian occurrence. The one failure may have been due to giving the drug at the wrong time. The malarias involved were 2 strains from the Pacific (1005-G and 1027-NG) and one from the Mediterranean (1031-Si). Five infections originated from blood transfer, and the remainder were induced by mosquito bites. Thus, the use of sodium bismuth thioglycollate to regulate the paroxysms of foreign *vivax* malaria seems to be quite dependable. A similar effect is observed when the drug is administered to patients infected with the St. Elizabeth strain of *P. vivax* (10).

Length of Primary Attack. Data on the number of paroxysms in 60 patients infected by mosquito bite were available and are shown in table 7.

Only 12 (20 per cent) cases terminated spontaneously. These averaged 8.5 paroxysms with the range extending from 4 to 14 paroxysms. The remainder (80 per cent) were treated to terminate the infections, some of which had shown 22 paroxysms.

TABLE 7
Number of Paroxysms in the Primary Attack (Mosquito Induced)

STRAIN	TERMINATION				
	SPONTANEOUSLY			BY TREATMENT	
	Number patients	Av. no. paroxysms	Range	Number patients	No. paroxysms range
1005-G	0	0	0	5	11-14
1019-G	0	0	0	3	11-13
1027-NG	8	7.5	4-14	26	9-20
1032-NG	3	9.7	8-11	4	11-14
1031-Si	1	13	13	10	8-22
Totals and averages.....	12	8.5	4-14	48	8-22

Of the 10 patients inoculated by blood with strain 1027-NG (which are not shown in table 7), only 2 (20 per cent) cases were self-terminated; one after 6 and one after 11 paroxysms respectively. Of those terminated by treatment, some had shown up to 22 fevers.

Heterologous Strain Immunity. Nine patients were given both native and foreign strains of *P. vivax* to determine whether heterologous immunity developed. These data are detailed in table 8.

Eight cases were first given the St. Elizabeth strain of *P. vivax*. The termination of the primary attack was spontaneous in these cases. In some cases parasitemias persisted after the last paroxysm, of which some were cleared by treatment before reinoculation. From 16 to 44 days after the last paroxysm of the primary attack, patients were inoculated with a foreign strain. All developed parasitemias and paroxysms of varying lengths, some of which (AMR, NB, JMD, and LG) approximated normal primary infections.

The ninth case (RSJ) was given first a foreign malaria which, after spontaneous termination, was followed by inoculation with the St. Elizabeth strain. The latter strain also produced a symptomatic infection consisting of 8 paroxysms.

The parasitemia at the first paroxysm of the second inoculation averaged higher than at the first paroxysm of the first inoculation. This might indicate some immunity following the first infection, particularly in those cases where the second parasitemias were many times higher (NB, LG, and AC). However, this pattern was not consistent as some of the fever threshold parasitemias of the second infection (OCJ and JBH) were lower than those of the first infection.

It is obvious, therefore, that the first infection did not produce enough immunity to prevent a different strain from developing subsequently. Furthermore, if any immunity was developed, it was not very effective in some cases from a parasitological viewpoint. This is similar to the results obtained by

TABLE 8
Heterologous Immunity. Foreign vs. Native Strains of P. vivax

PATIENTS	FIRST INOCULATION						SECOND INOCULATION						
	Strain	Method	No. parox.	Terminated	Parasites per cmm.		Day from last primary paroxysm	Strain	Method	No. parox.	Terminated	Parasites per cmm.	
					First parox.	Last parox.						First parox.	Last parox.
O. C. J.....	SE	M	6	S	1,708	308	16	1512-NG	M	7	S	62	712
J. M. D....	SE	M	2	S	6,750	5,850	42	78-NG	M	20	T	7,550	
N. B.....	SE	B	16	S	110	1,930	35	1512-NG	M	10	T	10,650	
A. C.....	SE	B	9	S		01,570	18	90-NG	M	7	S	3,150	140
L. G.....	SE	B	13	S	360	550	18	94-NG	M	11	T	10,350	
A. M. R....	SE	B	8	S	180	7,118	44	1512-NG	B	11	S	1,250	2,212
J. B. H....	SE	B	21	S	638	1,275	31	109-B	B	3	S	625	3,362
B. H.....	SE	M	17	S	725	7,075	31	1512-NG	B	5	S	11,750	9,250
R. S. J.....	109-B	B	12	S	75	1,312	38	SE	B	8	S	1,950	625
Total average.....			11.6		1,172	2,999				9.1		5,260	
Average for 6 whose second infection was spontaneously terminated.....			12.2		554	3,110				6.8		3,131	2,717

S—spontaneously; T—by treatment; M—transmitted by mosquitoes; B—transmitted by blood; SE—St. Elizabeth strain; 109-B—Burma strain; NG—New Guinea strain.

Kaplan *et al.* (5), who, using American and Pacific strains, found that “the reinfection of a previously *vivax*-infected patient with a heterologous strain would, on the average, produce clinical paroxysms totaling approximately 75 per cent of the clinical paroxysms experienced on original infection”.

DISCUSSION

Periodicity. Of the strains studied in detail, regardless of origin, none showed a 48-hour interval between fever peaks but, rather, a shorter periodicity. This is true of the five strains detailed in table 4, as well as a New Hebrides strain

reported earlier (8). Two American strains studied earlier also showed a shorter periodicity (8).

Kitchen (6) found the periodicity of the McCoy strain of *P. vivax* to be only 16 minutes short of 48 hours, whereas the periodicity of the strains, both native and foreign, studied by us averaged from 43.6 to 45.1 hours in length. However, there are points of difference in our observations. Apparently, most of Kitchen's observations were made on infections characterized by quotidian fevers; ours were made only upon fevers appearing every other day, either naturally or after conversion by administration of sodium bismuth thioglycollate. Furthermore, he found that the shorter cycles characterized rigorless paroxysms primarily and prevailed consistently only during the first week of the attack. As we waited for the conversion to the tertian periodicity, most of our readings were made during and subsequent to the second week of fever. As shown in table 6, chills accompanied the fevers less frequently during the first 5 fevers (about the first week) than after this time.

The difference in periodicities may be due to the fact that Kitchen measured quotidian fevers primarily, and we measured the tertian type only. It has been obvious in our work, even without measuring, that the tertian type of fever does not recur at 48-hour intervals, but shows a shorter periodicity.

The Use of Foreign Malarias as a Therapeutic Agent against Neurosyphilis. The foreign malarias tested appeared from a parasitological viewpoint to be satisfactory as a therapeutic agent in the treatment of white neurosyphilitic patients. Most of the white patients (95 per cent) became infected (9) after being bitten by infected mosquitoes. The prepatent and incubation periods were relatively short (table 1). Most of the infections (80 per cent) produced a satisfactory number of paroxysms (10 to 20). The infections which were self-terminated averaged 8.5 paroxysms, about the number of paroxysms desired by some clinicians. The parasite count seldom reached densities high enough to require drug intervention. The use of sodium bismuth thioglycollate was shown to be reliable in reducing the quotidian fevers to a tertian periodicity. Previous infection with native malarias seemed to produce little immunity against subsequent infections with these foreign strains. Other workers (4) using these same strains have shown that the infections responded promptly to adequate treatment.

But, as shown previously (9), these *vivax* malarias were not satisfactory in the treatment of Negro neurosyphilitics as most of these patients did not develop the infection.

SUMMARY AND CONCLUSIONS

1. White neurosyphilitic patients were infected with foreign *Plasmodium vivax* malaria, both by blood inoculation and by mosquito bite. Infections resulting from mosquito transmission showed the averaged prepatent period to be 12.8 days and the average incubation period to be 14.2 days. These periods were significantly shorter for the Mediterranean strains than for strains from the Pacific.

2. In the mosquito transmitted infections, the first maximum fever usually preceded the maximum parasitemia by several days. The average of the first maximum fever was 106.6° F. The maximum parasitemia averaged 13,900 parasites per cmm.

3. In the blood transmitted infections, the first maximum fevers and the maximum parasitemias usually occurred at about the same time. The maximum parasitemias were higher than in mosquito induced infections.

4. The tertian type paroxysms showed an average periodicity of 44.5 hours, ranging from 43.6 to 45.1 hours. None showed a 48-hour periodicity.

5. Chills accompanied the fevers in 73.2 per cent of the cases. Chills were less frequently present with the first 5 fevers than with the later fevers.

6. The types of fever at onset of mosquito induced infections were: quotidian, 50 per cent; remittent, 42 per cent; and tertian, 8 per cent. These types were often succeeded by a different type.

7. Sodium bismuth thioglycollate was reliable in changing remittent and quotidian paroxysms to tertian occurrence.

8. Usually, the primary infections produced over 10 paroxysms.

9. Little or no heterologous immunity was demonstrated between 5 foreign strains and the St. Elizabeth strain of *P. vivax*.

10. The foreign malarias appear to be satisfactory as a therapeutic agent to treat white neurosyphilitic patients. This was not true of Negro patients.

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THE MOLLUSCAN INTERMEDIATE HOST AND SCHISTOSOMIASIS JAPONICA

III. EXPERIMENTAL INFECTION OF *Oncomelania quadrasi*, THE MOLLUSCAN INTERMEDIATE HOST OF *Schistosoma japonicum*

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INTRODUCTION

The program of the Commission on Schistosomiasis included an attempt to determine the most successful method of maintaining colonies of the snail *Oncomelania quadrasi* (= *Schistosomophora quadrasi*) experimentally infected with *Schistosoma japonicum*. It was planned to expose uninfected, laboratory reared snails to determine the number of miracidia that would produce the highest infection and survival rates in the snails. Information was desired on the interval of time necessary for the production of cercariae after miracidia had penetrated the snail and also as to whether or not reproduction occurred among the snails kept under artificial conditions.

MATERIALS AND METHODS

After preliminary observations it became apparent that it was impossible to raise *O. quadrasi* in sufficient numbers or rapidly enough for use in the laboratory. Consequently, it was decided to select adult snails from areas in which infections with *S. japonicum* were absent or rare. Two such regions selected were the Limbujan and Kansanada-Kapohaun areas on Leyte, P. I. In all lots 200 snails were crushed after they had been kept isolated in aquaria and another 200 placed in an aquarium as a control. It was found that the infection rate in the 669 control snails examined by crushing was nearly 0.5 per cent (table 1). Consequently it was necessary to assume this as a base line and to superimpose significantly experimental infections.

Three methods were used in exposing snails. The first was to place an individual snail in a drop of water containing a known number of miracidia. When all the miracidia had disappeared exposure was considered complete. If any of the miracidia failed to attack the snail, they were removed and replaced by fresh miracidia. In this way, the snails were exposed to the desired number of miracidia. The disappearance of the larvae within a half hour was

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taken as presumptive evidence of their having penetrated the snail. However, it was impossible to determine accurately whether or not all actually penetrated even though infections were checked in some by dissection. A second method involved selecting a given number of snails and miracidia and placing them together in a predetermined ratio. The third consisted of adding indiscriminately hundreds of miracidia to aquaria containing many snails. Exposure was made in the same water (pH 7.6) in which the snails had been living and in which the eggs of *S. japonicum* had hatched.

The exposed and unexposed snails were placed in prepared aquaria. Some lots were housed in glass animal jars eight inches in diameter and eight inches high. These had a mud bottom and bank to which filtered river water (pH 7.6) was added to a depth of about two inches. Green vegetation was planted on the mud bank, and decaying nipa fronds and coconut bark strips were placed on the soil and in the water. Water was replenished regularly and the entire bottom of the aquarium was flooded occasionally. Once each day snails that had crawled up the glass wall were pushed back down. Three to four weeks after each aquarium was set up, the viable snails were counted and transferred to a new aquarium of similar type.

Starting with the sixth week, the snails were examined periodically to determine the number alive, the number positive or negative, and the cercarial yields of those found to be positive. Each batch of exposed snails was handled *en masse* until the cercariae first appeared. Thereafter the snails were isolated in order to facilitate the determination of positive snails as well as the individual cercarial yield. These experiments were concluded 66 to 78 days after exposure at which time all viable snails were crushed and examined to determine whether or not any cercariae or schistosome-like sporocysts were present (see table 1).

When the periodic examinations were begun the colonies of exposed and control snails were placed in terraria containing moist soil but no standing water. This was done to prevent the shedding of cercariae between examinations.

DISCUSSION OF RESULTS

Table 1 summarizes the results of our attempts to infect *O. quadrasi* with known numbers of miracidia of *S. japonicum* under laboratory conditions. It should be pointed out that these experiments could not be followed as carefully as planned due to the pressure of other experimental work, the departure of the senior investigator to the United States and the preparations necessary to the departure of the balance of the group to another area.

The three lots of control snails showed 7.5 to 15.5 per cent survival at the close of the experiments. Of this total of 600 selected as controls only 69, or 11.5 per cent, survived. It would seem that this figure should be regarded as the normal survival rate under laboratory conditions as they existed on Leyte.

Out of 1,321 snails individually infected with a known number of miracidia a total of 60 or 4.5 per cent survived for the 66 to 77 days that the experiment ran. It is obvious that none of these experiments was highly successful from

the standpoint of the numbers of survivors. However, these data are recorded in the hope that the results may be of some assistance to others working with snail infection problems.

Table 2 furnishes a comparison between the snails examined before the 70th day of the experiment and those checked subsequently. Six of 116, or 5.2 per cent, of all snails (including the controls) that were examined before the 70th

TABLE 1

Results of experimental infection of Oncomelania quadrasi with mass exposure and exposure to known numbers of miracidia of Schistosoma japonicum

EXPT.	NO. OF MIRACIDIA	NO. OF SNAILS USED	DAYS OBSERVED	NO. OF SNAILS ALIVE AT END OF EXPERIMENT	PERCENT OF SNAILS ALIVE	NO. OF SNAILS POSITIVE FOR CERCARIAE	PERCENT OF TOTAL NO. OF SNAILS POSITIVE FOR CERCARIAE	PERCENT OF SURVIVING SNAILS POSITIVE FOR CERCARIAE
Controls								
41A	0	200*	65	15	7.5	0	0	0
41B	0	200*	65	23	11.5	1	0.5	4.3
41C	0	200*	65	31	15.5	1	0.5	3.2
Individual exposures								
40.1	1	205	67	3	1.5	0	0	0
43	3	211	66	16	7.6	0†	0	0
44	3	201	68	3	1.5	0	0	0
38.1	5	298	77	6	2.0	2	0.7	33.3
39.1	8	205	75	7	3.4	2(1)‡	0.97	28.5
42	10	201	66	25	12.4	4(10)	2.0	16.0
Mass exposures								
38.2	Aver. 5	909	78	1	0.1	1	0.1	100
39.2	Aver. 8	527	78	2	0.4	2	0.4	100
38.3	Mass	875	77	8	0.9	8	0.9	100
38.4	Mass	825	73	0	0	0	0	0
39.3	Mass	150	66	0	0	0	0	0

* An additional 200 were crushed as controls and were found to have the same infection rate.

† Infections present four days previously.

‡ Additional specimen(s) with schistosome-like sporocysts.

day were positive for cercariae of *S. japonicum*. Excluding the controls, four of 47 were positive. These figures are in sharp contrast to the presence of 15 or 62.4 per cent, positive in the 24 examined *after* the 70th day. Although these differences are significant, a satisfactory explanation for the phenomenon is not at hand.⁵

Snails exposed to one or three miracidia each did not have as high a survival

⁵ The assistance of Dr. R. L. Gauld is gratefully acknowledged.

rate as those exposed to larger numbers. Thus a total of 22 out of 617, or 3.5 per cent, survived. Furthermore, none of these surviving snails were infected when they were crushed at the end of 66 to 68 days. It should be noted that this survival rate was only slightly less than the 4.5 per cent found as the average for all 1,321 individually infected snails. If the 617 snails exposed to one or three miracidia are removed from the calculations, 5.4 per cent of the remaining 704 survived.

Only six of the 298 snails exposed individually to five miracidia were alive at the end of 77 days when this experiment was concluded. However, two of the survivors were positive for cercariae of *S. japonicum*.

Seven of 205 of the snails exposed individually to eight miracidia survived the 75 days that this experiment was carried on. Two of these survivors yielded cercariae. In addition, another contained schistosome-like sporocysts when crushed.

TABLE 2

Comparison of snails examined before and after the seventieth day of the experiment

	NO. OF SNAILS	NO. OF SNAILS POSITIVE FOR CERCARIAE	PERCENT OF SNAILS POSITIVE FOR CERCARIAE
All Snails Examined Before 70th Day (Including controls)	116	6	5.2
Experimentally Infected Snails Examined Before 70th Day	47	4	8.5
Experimentally Infected Snails Examined After 70th Day	24	15	62.4

In contrast to this, 25 of the 201 snails exposed to 10 miracidia each survived the 66 day experimental period. Four of these were positive for cercariae of *S. japonicum* while 10 others contained sporocysts. While these data are by no means conclusive, they suggest that for some unknown reason a higher survival rate was obtained with snails exposed to 10 miracidia each. The principal variables in this experiment were the number of miracidia to which the snails were exposed, the age of the snails, and death rate of different lots of snails. While measurements were not made on many of the snails selected for the experiments, most were probably young adults as they ranged between 2.75 and 4.0 mm (McMullen 1947). Hence it does not seem likely that these experimental snails died of old age. Furthermore there is no evidence that the infection itself killed the snails, as the highest death rates occurred in the groups exposed to the fewest miracidia.

Table 1 summarizes the results obtained from the mass infections. All of these showed a low survival rate, even markedly lower than the other infected snails. It is possible that in the experiments where excessive numbers of miracidia were made available (as in the mass experiments with unknown quantities of miracidia) many of the snails received overwhelming infections and so

did not survive. Of five such groups all snails in three groups that survived the period of observation for 77 to 78 days were positive for cercariae of *S. japonicum*.

Nineteen of the 71 snails that survived experimental infections until the snails were crushed were positive for cercariae. This should be compared with the control snails where 2 of 69 were positive at the end of the experiment. In addition, another 11 snails, on crushing, were positive for schistosome-like sporocysts. If those with sporocysts but without mature cercariae in the experimentally infected snails had been included, the percentage of positives would have been raised considerably. Those with sporocysts present were in various developmental stages, most containing immature fork-tailed cercariae. Two of the snails containing sporocysts without mature cercariae had been exposed 66 days previously. One group of snails which had not shed cercariae through the 73rd day began shedding on the 77th day while two other groups produced cercariae for the first time on the 78th day. It would seem that eleven weeks is approximately the minimum period required for the development of mature cercariae under the conditions in which the snails were held in the laboratory. These observations are in essential agreement with those of Faust and Meleney (1924) for *S. japonicum* in the intermediate host in China.

It was hoped that data would be secured on the number of cercariae produced from a single miracidium as well as the number of days over which shedding takes place. However, none of those exposed to a single miracidium that survived was infected.

The high mortality rate shown by the entire series including both controls and experimentally infected snails suggests that proper ecological conditions were not established. Influencing factors may have been overcrowding, absence of direct sunlight, improper food and too high a water temperature. Apparently the best results were secured in an aquarium constructed from a longitudinally split oil drum which was seeded with adult snails of *O. quadrasi* about two months prior to the beginning of these experiments. Here the water was maintained at a pH of 7.6. Although no snail eggs were ever observed in it, young specimens of *O. quadrasi* were recovered from the aquarium five months later. It was determined by Dr. D. B. McMullen that these snails were too young to have developed from eggs which might have been present in the muck originally introduced into the terrarium. It is virtually certain that this muck did not contain eggs of *O. quadrasi* as the soil was taken from an area where these snails had not been found. It must be concluded, therefore, that these young were the product of snails breeding in this artificial environment and so constitutes one of the first records of the reproduction of *O. quadrasi* under laboratory conditions. In another aquarium similarly maintained but kept in an acid condition no reproduction was observed.

CONCLUSIONS

1. In this series of experiments the best results were secured by exposing each snail individually to from five to ten miracidia.

2. The evidence secured indicates that a period of approximately eleven weeks is required for the development of cercariae of *S. japonicum* in *O. quadrasi* under the laboratory conditions to which the snails were subjected on Leyte, P. I.

3. Four of 47 snails examined before the 70th day of the experiment were positive in contrast to 15 of 24 of those examined subsequently.

4. All snails surviving mass infection were positive but the mortality rate in this group was extremely high.

5. Reproduction of *O. quadrasi* was noted to have occurred under laboratory conditions.

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THE RECTOSCOPIC BIOPSY BY TRANSPARENCY. A NEW DIAGNOSTIC METHOD FOR SCHISTOSOMA MANSONI

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The rectoscopic biopsy is a new clinical method devised to increase the diagnostic frequency of infections of *Schistosoma mansoni*. It was published in an earlier paper with Dr. H. Atencio M. in 1943 (1). At the time of the appearance of this paper the diagnostic percentage obtained in Caracas, Venezuela with stool examinations ranged from 2 to 9 per cent depending on the number of examinations performed on each patient, and the type of human material examined (outpatients, hospital, or sanatorium patients). The diagnostic percentage in autopsy material examined histologically was about 20 per cent.

The rectoscopic biopsy method is based on certain fundamental facts that we elicited while studying autopsy material. By digesting with caustic potash according to the method recommended by Fergusson (2), and then examining the sediment microscopically we demonstrated a 45 per cent infection with *S. mansoni* in liver fragments taken from autopsies. Following a similar procedure we found a 60 per cent infection by examining tissue from the upper half of the rectal ampulla. These figures were much higher than those obtained either with stool examinations in living patients, or with previous histological methods used on autopsy material. Therefore, the biopsy of a fragment of rectal tissue, taken from the living patient, and digested with caustic potash, should give positive results two to three times higher than those previously obtained from histological examination of autopsy material. Also, the biopsy of a fragment of rectal tissue digested with caustic potash shows eggs more frequently than a biopsy of liver material treated in the same way. In addition the biopsy of liver material is a much more dangerous procedure than as stated in our first paper.

TECHNIC OF RECTOSCOPIC BIOPSY

(1) Punch out with a biopsy forceps a fragment containing mucous and submucous tissue from the upper half of the rectal ampulla, taking the material from the middle Houston valve at about 10 cm. from the anus in the area drained by the superior hemorrhoidal veins. (2) Digest this tissue fragment for three to four hours in a four per cent caustic potash solution at a temperature of 60–80°C, and after centrifugation examine the sediment microscopically.

In order to submit the rectoscopic biopsy method to a hard test we selected for examination a group of people who from clinical and epidemiological evidence appeared to be very lightly infected with *S. mansoni*. In such a group we found in 1943 (1) 20 per cent positive. In 1945 Dr. Rincon Urdaneta (3) in his Doctor's Thesis tested the rectoscopic biopsy in 128 suspected patients and found 42.2 per cent positive. Therefore, of 42 positive cases that we can now

detect among 100 suspected patients, 33 to 40 would have been missed had we only resorted to stool examinations.

It, also, seems reasonable to draw from the figures given above the conclusion that the clinical knowledge on schistosomiasis in Venezuela needs a thorough revision. This clinical knowledge has been based on the small group of cases with positive stool tests, 2 to 9 cases among 100 suspected. But the large group of infected cases, 33 to 40 among 100 suspected, with negative stool test, remained unknown clinically. The rectoscopic biopsy, thus, opens a new field for the clinical study of this disease.¹

Moreover, the work of Dr. Rincon Urdaneta (3) is particularly interesting because it presents a group of 67 cases comparatively studied with stool test, rectoscopic biopsy, and intradermal test. The latter was performed with antigen prepared from adult worms of *Schistosoma mansoni*. The following table shows the results arrived at with each method:

	CASES	POSITIVE	PER CENT POSITIVE
Stool tests.....	64	5	8
Biopsies.....	67	33	49
Intradermal tests.....	67	39	58

The high positive percentage obtained with the rectoscopic biopsy is remarkable. It is a direct method with only a negative error (false negatives). When compared with the intradermal test results it has only a 9 per cent difference. The intradermal test has both positive and negative errors (false positives and false negatives) as all indirect methods have. Before we had the rectoscopic biopsy it was impossible to have a scientific control on the results of the indirect methods.

If we now analyze further the results given by Dr. Rincon Urdaneta we see that both biopsy and intradermal tests were positive in 24 cases. In 9 cases the biopsy was positive and the intradermal test negative. Thus the intradermal test's negative error (false negatives) in this series was 13 per cent. In 15 cases the intradermal test was positive and the biopsy negative. Thus, the intradermal test's positive error (false positives) seems to be 22 per cent. But we must keep in mind that the biopsy method has also a small negative error, so that we cannot charge the whole figure of 22 per cent to the intradermal test's positive error. The remaining 19 cases in this series were negative by both methods. This comparison certainly emphasizes the value of the rectoscopic biopsy method as a reliable procedure to control the results of the indirect methods.

The chief aim of the present paper is to present certain improvements in the rectoscopic biopsy method which will make it simpler to carry out and at the same time will reduce the number of false negatives. That such negatives

¹ The results given by Hernandez-Morales and Maldonado from Puerto Rico, in a paper which appeared in November 1946 in this Journal, confirm also our conclusions.

exist is shown by the finding of Dr. Rincon Urdaneta of three cases giving a negative biopsy but a positive stool test. In our earlier paper (1) we suggested that multiple biopsies of specimens taken simultaneously or successively at different levels on the rectal ampulla may be very helpful in reducing this error.

Also in an endeavor to simplify still further the rectoscopic biopsy technic, another factor was found which may have an influence on the negative error. Direct microscopical examination of the fragments of rectal tissue, compressed between a slide and cover glass, was made before caustic potash digestion was used, to try to see the eggs immediately and thus to avoid further manipulation of the specimen. In heavy infections it is very easy to see the eggs in such preparations toward the epithelial layer, or in the interglandular tissue, but when the eggs are scarce it is difficult to find them in such preparations, and, therefore, in our first paper (1) the caustic potash tissue digestion method was advocated as preferable to direct examination. Many devices were tried unsuccessfully to make the tissue fragments transparent before a very simple but satisfactory one was found. By immersing the tissue fragments in 5 cc. of fresh water for a period of three to five minutes the hemoglobin is easily dissolved, and the fragments become quite transparent, the eggs appearing in characteristic strings. Furthermore, the living fragment absorbs water quickly and after three to five minutes becomes a watery globule, which, when compressed between two slides, becomes a thin transparent film. The eggs can be easily detected in this film by focusing with the low power at different levels. These observations led to the development of the following technic for the diagnosis of *S. mansoni*.

RECTOSCOPIC BIOPSY BY TRANSPARENCY

(1) Take rectal tissue fragment with the biopsy forceps as previously described. (2) Immerse fragment in fresh water for three to five minutes; compress the hydrated tissue globule tightly between two slides; add a drop of water to the transparent tissue film; cover, press gently, and examine with the low power of the microscope.

This method simplifies and reduces the procedure to a few minutes work. Furthermore, it is more reliable and dependable than the caustic potash digestion method in very light infections. Extensive studies of many fragments containing only a few eggs has shown that when only one or two eggs are imbedded in the tissue fragment they are easily lost in the caustic potash digestion. They may adhere to the centrifuge tube or to the pipette, or may be thrown away with the supernatant fluid. Thus, cases of this type will be positive by the transparency method but negative by the digestion method.

The transparency method can also be used with fragments of rectal tissue from autopsy cases. But since large fragments of tissue can be easily obtained at autopsy the caustic potash digestion method is usually preferable.

When examinations of the tissues are made by the transparency method striking differences are found among the eggs in the same fragment and even in

the same microscopic field. Some eggs appear normal, golden yellow in color, and contain moving miracidia; others are quite small and nearly black with abnormal contents; a few are a little larger than these, not so dark colored but with numerous black inclusions and no miracidia. In addition, eggs are seen which appear nearly normal, but which contain a few inclusions and vacuoles, and in which the miracidia appear to be dead. In spite of the presence of these abnormal eggs, our observations show that very commonly normal eggs containing living miracidia are present in the wall of the rectum of untreated patients. Investigators have repeatedly stressed the importance of being able to find normal eggs with living miracidia in relation to the advisability of treatment procedures. For this reason Fülleborn (4) avoided the use of concentration methods that kill the miracidia. The caustic potash digestion method also kills the miracidia. In consequence, it does not permit to establish the therapeutical indication on a sound basis although we secure through it the diagnosis of *Bilharzia* infection beyond any doubt.

The method of rectoscopic biopsy by transparency has the advantage of making it possible to determine whether the miracidia in the eggs are alive. It, therefore, can be used to establish the advisability of treatment on a sound parasitologic basis, and to check the results of treatment. It is not difficult to examine individual eggs with a high power to detect movement of the miracidia. It is even possible without too much difficulty to uncover the preparation, and with a fine needle, puncture an egg to deliver the miracidium under microscopic control.

CONCLUSIONS

1. The method of rectoscopic biopsy by transparency permits a clinical diagnosis of *Schistosoma mansoni* infection in a few minutes.
2. The high figures already obtained with the rectoscopic biopsy alone suggest a thorough revision of our clinical knowledge of schistosomiasis.
3. The rectoscopic biopsy by transparency enables a demonstration of eggs with living miracidia, thus securing positive evidence in favor of a medical treatment.

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FILARIAL INFECTION IN COSTA RICA

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Puerto Limon, situated on the Atlantic coast of Costa Rica at 10° North latitude, has an estimated population of 12,000 people. For the purpose of this study it has been conveniently divided into the following four sections or areas: Puerto Limon proper, Hospital Point (the American and British Colony), Jamaica Town and Cieneguita (Fig. 1).

This survey was started early in 1946 in the Cieneguita section which is separated from Limon proper by the Cieneguita River, and consists of a narrow strip of land between the River and the Atlantic Ocean (Fig. 2). Near the extreme end of this strip is the airfield used by the commercial air lines operating between Costa Rica and Panama. The population of this section was 706 at the time of our survey.

Jamaica Town, recently renamed Barrio Roosevelt, lies directly across a roadway from the American and British Colony in which is located this Hospital and the residences of several employees of the United Fruit Company (Compania Bananera de Costa Rica) and the Northern Railroad. The estimated population of Jamaica Town is 2,000 people. Of this number only 300 individuals have been studied to date. The location of the houses in Jamaica Town which are included in this report, and their proximity to Hospital Point is shown in Fig. 3.

All blood smears were made between the hours of 10 P.M. and 6 A.M., the great majority between 10 P.M. and 2 A.M. Assisting me in this house to house survey was the Local Sanitary Inspector placed at my disposal by the Governor of Limon Province, and alternating members of my local laboratory staff. To all of these I wish to express my sincere appreciation. Without their assistance such a survey would have been almost impossible.

All data regarding age, place of birth, length of residence in Limon, and other pertinent facts were obtained during the initial visitation. As many of the dwellings in both areas were almost inaccessible following heavy rains, our progress was slower than I had hoped due to the unusually heavy rainfall during several months of this survey. The total rainfall in Limon for June, July and August was 83.09 inches, the heaviest recorded in the past 10 years.

In the Cieneguita and Jamaica Town sections combined, 269 houses were surveyed and a total of 1,006 blood smears were made and examined for both microfilariae and plasmodia.

As the question was later raised as to the possibility that we were dealing with infections imported from Cuba, Jamaica, or various other islands of the British West Indies, I have confined the studies, subsequent to the establishment of infection, to those individuals who were born and have lived their

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entire lives in Limon. Of the total of 101 positive smears for microfilariae found in the Cieneguita and Jamaica Town sections, 53 (52.4 per cent) were

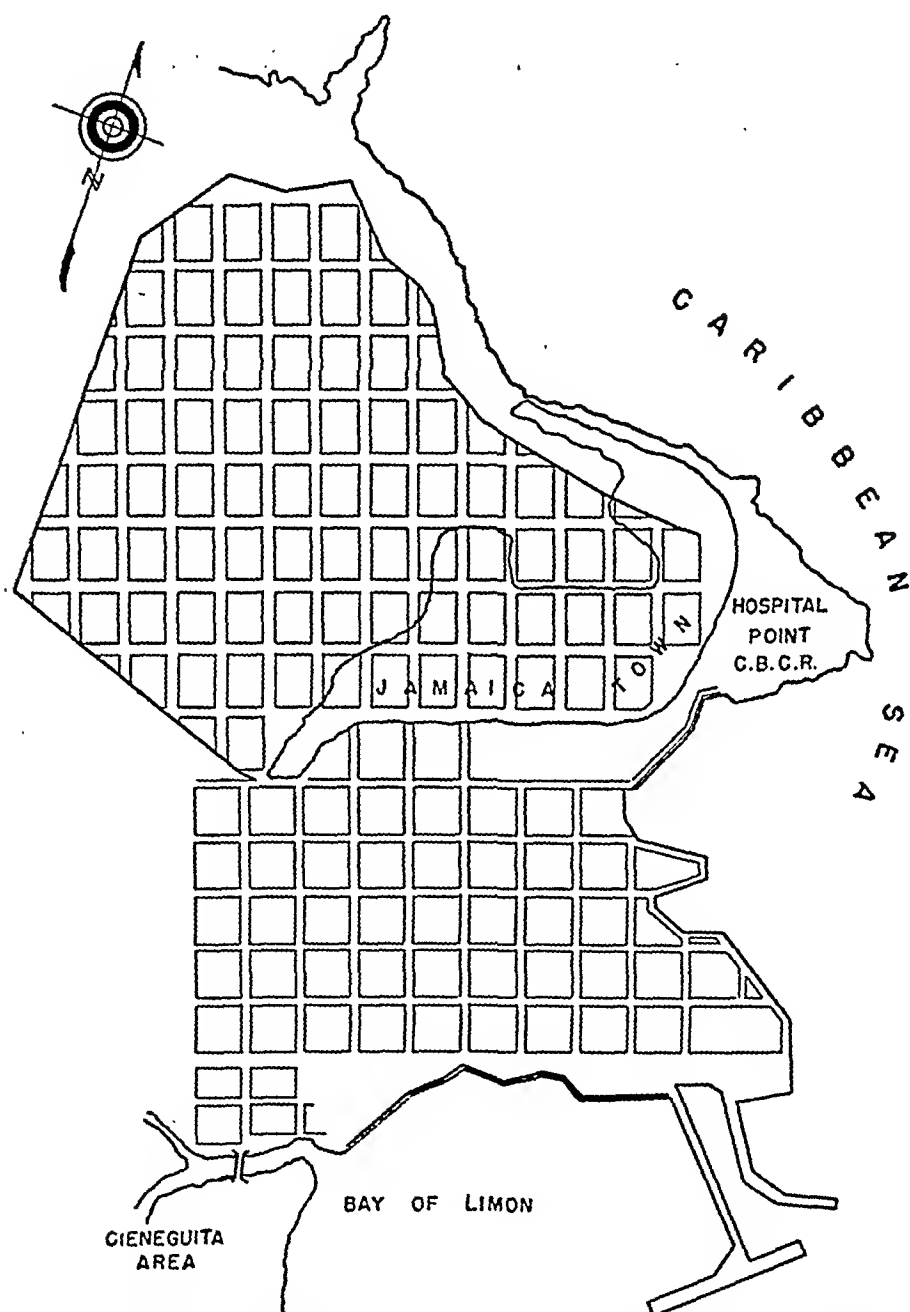


FIG. 1. City of Limon, Costa Rica. *Puerto Limon*. Hospital Point, Jamaica Town and Cieneguita areas indicated.

natives of Limon. The complete analysis of data on these individuals is found in table 1.

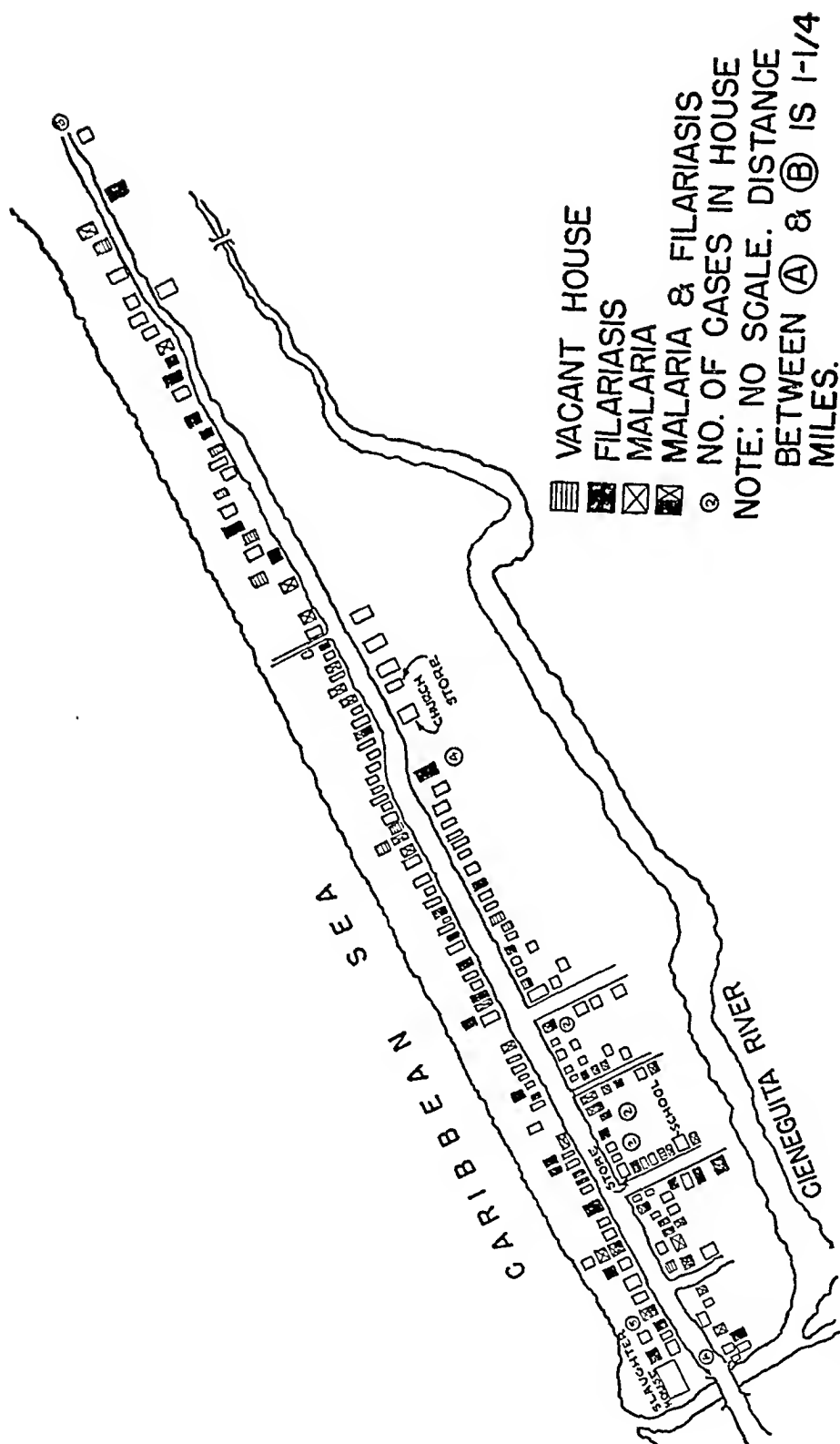


Fig. 2. Filariasis and Malaria Survey. Cieneguita Area. Puerto Limon, Costa Rica 1946. Cieneguita Section of Puerto Limon, showing location of infected persons.

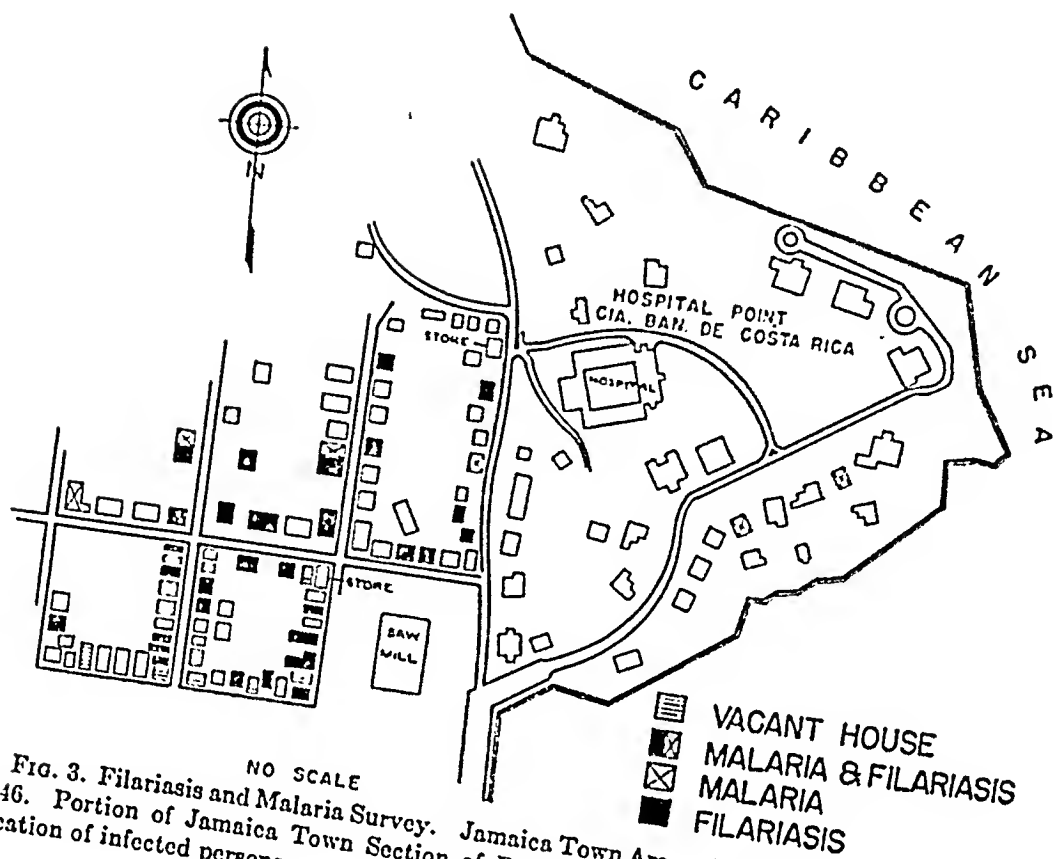


FIG. 3. Filariasis and Malaria Survey. Jamaica Town Area. Puerto Limon, Costa Rica 1946. Portion of Jamaica Town Section of Puerto Limon included in survey, showing location of infected persons.

TABLE 1
FILARIASIS SURVEY
Costa Rica—1946

TABLE 1

FILARIASIS SURVEY

Costa Rica—1946

AREA SURVEYED	NUMBER OF SMEARS EXAMINED	DISTRIBUTION ACCORDING TO RACE & SEX								TOTAL NUMBER OF POSITIVE SMEARS	PERCENT	NO. OF INFECTED LIVING IN LIMON AREA FOR TWO YEARS OR MORE	DISTRIBUTION OF INFECTED PERSONS ACCORDING TO RACE & SEX								NO. OF INFECTED PERSONS LIVING ENTIRE LIFE IN LIMON AREA			PERCENT OF TOTAL POSITIVE SMEARS	
		White		Negro		Mixed		Chi-nese					White		Negro		Mixed		Chi-nese		White	Negro	Mixed		
		M	F	M	F	M	F	M	F				M	F	M	F	M	F	M	F	White	Negro	Mixed		
Limon Cieneguita Area	700	85	96	232	274	8	7	3	1	56	7.0	56	4	2	27	23	0	0	0	0	4	26	0	53.6	
Limon Jamaica Town	300	12	10	119	149	6	3	1	0	45	15.0	45	0	0	16	25	3	1	0	0	0	19	4	51.1	
Bataan	100	39	19	23	19	0	0	0	0	3	3.0	3	0	0	1	2	0	0	0	0	0	0	0	0	4.0
Quespos	100	50	47	0	0	0	3	0	0	1	1.0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Cahuitta	100	6	2	53	37	0	2	0	0	2	2.0	1	0	0	2	0	0	0	0	0	0	0	0	0	0
Total	1,306	192	174	427	479	14	15	4	1	107	10.6	106	5	2	44	52	3	1	0	0	4	45	4		

Although all authorities agree that *Wuchereria bancrofti* infection is widespread throughout tropical and subtropical areas, I have been unable to find any specific reference to its occurrence in Costa Rica. That the microfilariae with which we were dealing were sheathed was apparent from the very start of the survey; however, I am indebted to Dr. Ernest C. Faust of Tulane University and to Dr. H. W. Brown of Columbia University for substantiating this observation. As *W. malayi* and *Loa loa* are not known to exist in the Western Hemisphere, there is little, if any, doubt but that the microfilariae

TABLE 2

FILARIASIS SURVEY

Costa Rica—1946

Analysis of findings obtained during physical examination and diurnal blood smears on 19 infected persons

(Note: 16 of these showed a definite eosinophilia)

CASE NO.	SEX	COLOR	AGE	PLACE OF BIRTH	LENGTH OF RESIDENCE IN LIMON	NUMBER OF MICROFILARIAE IN NOCTURNAL SMEAR	NUMBER OF MICROFILARIAE IN DIURNAL SMEAR	FEVER	CHILLS	HEADACHE	CHRONIC COUGH	VOMITING	LYMPHADENOPATHY	PLASMODIA	REMARKS
1.	F	N	18	Limon	Life Time	3	0	-	-	-	-	++	+	-	Chyluria
2.	F	N	8	Limon	Life Time	1	0	+	+	++	-	+	+	-	
3.	F	N	30	Limon	Life Time	20	0	-	-	+	-	+	+	-	
4.	F	N	20	Limon	Life Time	2	0	-	+	-	+	-	+	-	
5.	M	N	9	Limon	Life Time	3	0	-	-	+	-	-	+	-	
6.	F	N	33	Limon	Life Time	3	0	+	-	+	+	-	++	-	
7.	M	N	15	Limon	Life Time	8	0	-	-	-	+	-	++	-	
8.	F	N	31	Limon	Life Time	10	1	-	-	+	-	-	+	-	
9.	F	N	8	Limon	Life Time	4	0	-	-	-	-	-	+	-	
10.	F	N	33	Limon	Life Time	1	0	-	-	-	-	-	+	-	
11.	F	N	9	Limon	Life Time	2	0	+	-	+	-	-	++	-	
12.	F	N	27	Limon	Life Time	4	0	+	+	+	-	+	+	-	
13.	M	N	41	Limon	Life Time	28	0	-	-	-	+	-	+	-	
14.	F	N	15	Limon	Life Time	1	?	-	-	++	+	-	+	-	Slight edema of both legs
15.	F	N	12	Limon	3 Years	3	0	?	?	?	?	?	+	-	
16.	M	N	70	B.W.I.	50 Years	16	3	+	-	+	-	-	+	-	Slight edema of both legs
17.	F	N	42	Jamaica	40 Years	3	0	+	-	+	-	-	+	-	
18.	F	W	80	Nicaragua	50 Years	5	0	+	+	+	+	-	+	-	
19.	F	N	23	Costa Rica	10 Years	1	0	+	+	+	-	+	-	-	

observed were *W. bancrofti*. The nocturnal periodicity displayed by them adds additional confirmation as to their identity (see table 2).

Due to circumstances beyond our control, physical examination of those persons found positive for filarial infection has been extremely slow. To date, only 19 of the 53 native-born individuals have been examined. The results of individual examinations is shown in table 2. It will be noted by reference to table 2 that malaria may be ruled out as being responsible for certain of the recorded signs and symptoms. However, the high percentage of eosinophilia observed in the majority of these cases may be attributed to helminthic in-

fections, as routine stool examinations made in our laboratory at the Limon Hospital show more than 50 per cent positive for one or another protozoan or helminthic parasite. I am indebted to Dr. Oscar Pacheco, Medical Superintendent of the Limon Hospital for authorizing, and to Dr. A. González-Luján for making, the physical examinations herewith reported.

At the suggestion of Dr. Herbert C. Clark, Director of the Gorgas Memorial Laboratory in Panama, with whom I discussed this entire survey, a similar study was carried out at Bataan. This abacá farm, with a population of 1,500 to 2,000 people lies about 9.5 miles inland from the coast and is 25 miles by railroad from Limon. The majority of the population are native Costa Ricans from the inland towns of Cartago, Siquirres and Turrialba and have never resided in Limon. Being a company farm it allowed for the minimum loss of time in obtaining blood specimens, due to the close proximity of the living quarters. Of the 100 individuals examined here, 37 had lived in Limon for one year or more. Of this number, 3 (8.1 per cent) were found positive. These three individuals had lived in Limon for 3, 10 and 25 years respectively. In no case was there found a person infected who had never lived in Limon.

Our next survey was made at Quepos on the Pacific coast of Costa Rica. Here also 100 blood smears were made and examined. The population here is predominantly white, and only 17 of those studied had lived for one year or more in Limon. Of these, only one was found infected with microfilariae. This was a 15 year old white boy who had lived for 9 years in Limon.

The final study included in this survey was made at Cahuita, a small coastal town 25 miles southeast of Limon (Fig. 4). The population of this town, which does not exceed 200 persons, is predominantly negro. In our survey here, 47 dwellings were visited and 100 blood smears were made. Of these 100 persons, 8 were native whites, 2 mixed, and 90 were negroes. Only 2 persons were found infected with microfilariae. One was a 40 year old negro woman who had lived 23 years in Limon; the other, a 25 year old negro girl who was born in Cahuita and had never been to Limon. It appears logical to speculate, due to the close proximity of the two dwellings (Fig. 4) that infection of the latter case could be traced to the Limon resident.

In order that the vector of the infection in the Limon areas might be tentatively determined, the collection of larvae and adult mosquitoes from the yards and houses of infected native-born individuals was undertaken. I was assisted in this collection by Mr. Samuel D. Macready, General Sanitary Inspector of the Costa Rican and Panamanian Divisions of the United Fruit Company.

A total of 213 larvae and 123 adult mosquitoes was collected. Of the larvae collected, 173 (81.2 per cent) were *Aedes aegypti* and 27 (12.7 per cent) were *Culex quinquefasciatus*. The complete identification of the adults is shown in table 3. I am indebted to Dr. W. H. W. Komp of the United States Public Health Service for the identification of all larvae and adults, and for his suggestions regarding future work along this line.

It is of interest to note that in the Jamaica Town section, where the incidence

of filarial infection is nearly double that of the Cieneguita section, that the predominating species of mosquito was *C. quinquefasciatus*, the outstanding proven vector of the disease. It is hoped that through transmission experiments which are planned for the future that the possibility of other local species of mosquitoes may be definitely determined as vectors of *W. bancrofti* infection.

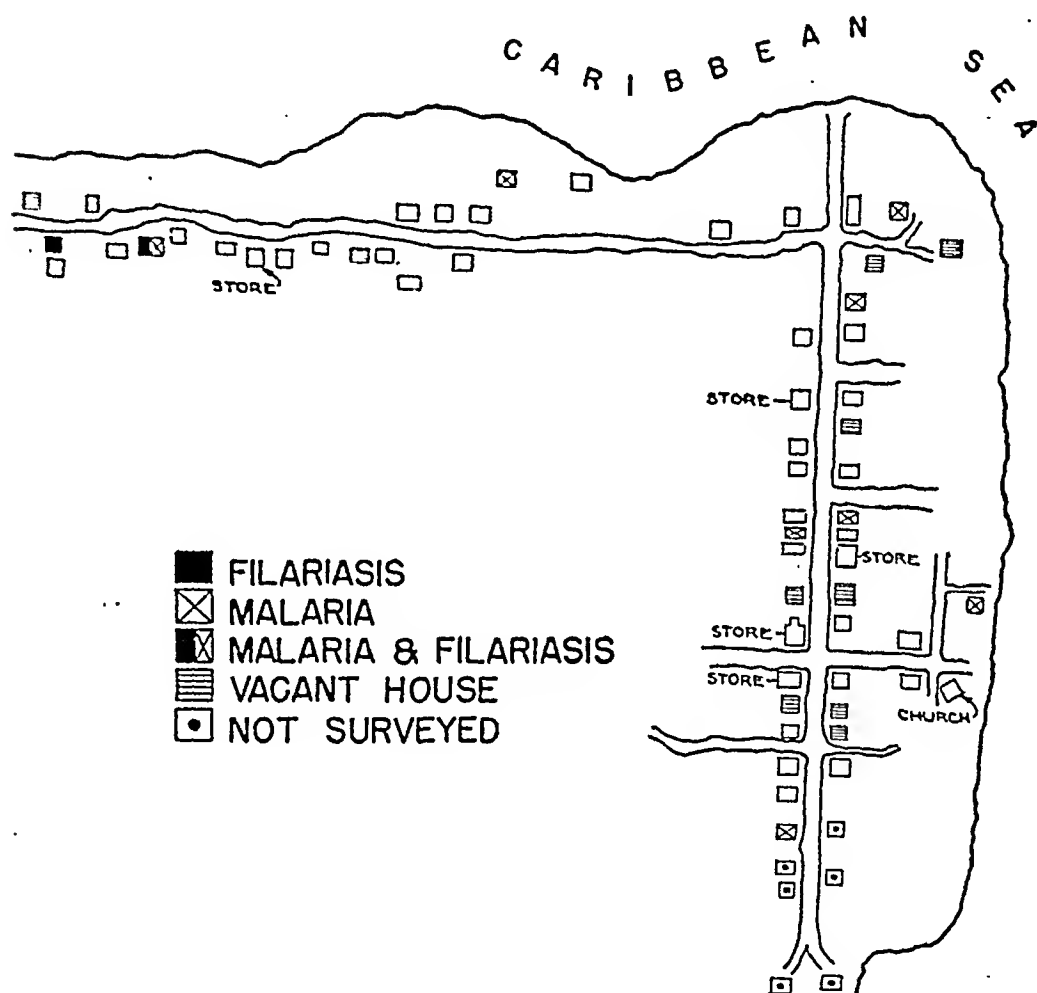


FIG. 4. Filariasis and Malaria Survey. Cahuita, C. R. 1946.

As already stated, all thick films were also examined for plasmodia. The analysis of these examinations is shown in table 4.

Before summarizing the pertinent data obtained from this survey, I wish to express to Dr. Edward I. Salisbury, Medical Director of the United Fruit Company, my deep appreciation for his support and encouragement throughout this entire study, and to Mr. G. D. Wood for preparing from relatively crude maps and tables the illustrations which accompany this report.

TABLE 3

ADULT MOSQUITO SURVEY

Jamaica Town & Cieneguita area Puerto Limon, Costa Rica—1946

	CULEX QUINQUE- FASCIATUS		CULEX MELANO- CONION		CULEX CHRYSO- NOTUM		CULEX UNCLAS- SIFIED	AEDES AEGYPTI		MANSONIA TITILLANS		DEINOCER- ITES SP	
	♀	♂	♀	♂	♀	♂		♀	♂	♀	♂	♀	♂
Jamaica Town	50	2	13	0	5	0	6	1	0	1	0	1	0
Cieneguita area	5	1	2	2	4	1	1	9	4	1	0	3	1
	CULEX LATISQUAMA		ANOPHELES ALBIMANUS		ANOPHELES NEOMACU- LIPALPUS		ANOPHELES VESTITIPENNIS		ANOPHELES PUNCTIMACULA		AEDES TAENIO- RHYNCHUS		
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
	0	1	3	0	1	0	3	0	1	0	1	0	

Jamaica Town

Total houses checked.....	11
Total adult mosquitoes collected.....	79
Total culex quinquefasciatus.....	52-65.8%

Cieneguita area

Total houses checked.....	18
Total adult mosquitoes collected.....	44
Total culex quinquefasciatus.....	6-13.6%

TABLE 4

FILARIASIS & MALARIA SURVEY

Costa Rica—1946

Relative incidence of filarial infection and malaria in surveyed areas

AREA	NUMBER OF SMEARS	MALARIA		FILARIAL INFECTIONS
		PERCENT POSITIVE		
		Falciparum	Vivax	Percent positive
Cieneguita	706	2.0	4.7	7.9
Jamaica Town	300	0.33	2.3	15.0
Bataan	100	0.0	2.0	3.0
Quepos	100	2.0	2.0	1.0
Cahuita	100	8.0	4.0	2.0

SUMMARY

A survey of 1306 persons from four areas of coastal Costa Rica has been made for the purpose of determining the presence and distribution of individuals

infected with microfilariae. That these microfilariae are *W. bancrofti* is born out by the fact that they were definitely sheathed and of nocturnal periodicity. The identification has been verified by two outstanding authorities, Drs. Faust and Brown.

The incidence of infection ranged from 1 per cent on the Pacific Coast to 15 per cent in certain areas on the Atlantic Coast. This would definitely establish certain portions of Costa Rica as regions of moderate infestation.

Among the native born population studied, the incidence was considerably higher among the negroes than among the whites.

The age incidence among the native born individuals is as follows:

Age	Percent infected
1-10.....	18
11-20.....	36
21-40.....	36
41-60.....	10

The youngest infected native born individual was 4 years of age and the oldest was 55 years.

From our studies made throughout Costa Rica it appears that Limon was the original endemic focus of the infection. That it was originally imported into this country from Jamaica and the West Indies there is very little doubt. That with migration of infected individuals from the Limon area to other parts of Costa Rica there is likewise little doubt the infection will become endemic throughout Costa Rica unless rigid mosquito control is put into effect.

It appears probable, from the preliminary entomological studies, that *Culex quinquefasciatus* is the vector of filarial infection in the areas studied. This opinion is shared by Dr. Komp. However, proposed transmission experiments may incriminate other species which are known to exist in the endemic area.

REACTIONS OF NATIVES OF OKINAWA AND OF AMERICAN PERSONNEL, TO SKIN TESTS WITH TEST ANTIGEN PREPARED FROM MICROFILARIAE OF *DIROFILARIA IMMITIS*

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U. S. Naval Medical Research Unit No. 2

Received for publication January 17, 1946

Several publications (1, 2, 3, 4, 5) have dealt with the use of test antigens prepared from the adult heart worm, *Dirofilaria immitis*, of the dog for making skin tests in patients with filariasis. These antigens were found to give positive dermal reactions in the majority of suspected cases of filariasis, but, because of cross reactivity in patients infected with other helminths, the negative responses in particularly severe infections and the equivocal results in subclinical cases, are of doubtful aid in the diagnosis of the individual case.

Canning (6), testing the various organs of the roundworm, *Ascaris lumbricoides* var. *suum*, for their antigenicity and specificity, showed the female reproductive tissue to be more specific than that of the male. Oliver-Gonzalez (7) demonstrated that, in the sera of rabbits infected with embryonated *Ascaris* eggs, the anti-egg antibodies were the most lasting and gave higher precipitin titers. The antigenic behavior of the embryos of filarids has received considerably less attention, probably because of the difficulties that attend their separation from the blood. However, the work mentioned above suggests that they may be a reagent of increased specificity, and, therefore, that reactions to them may possess considerable diagnostic significance.

Bruynoghe (8) was unable to demonstrate positive serological tests or skin tests in guinea pigs and rabbits after having prepared them by the introduction of microfilariae into the peritoneum. Acton and Rao (3) failed to obtain positive intradermal reactions with embryos of *Wuchereria bancrofti*. In both these instances it is likely that the antigen extracts were too weak. Using an antigen which was a mixture of microfilariae of *W. bancrofti* and white blood cells, Oliver-Gonzalez and Bercovitz (9) demonstrated precipitin antibodies in the sera of filarial patients. Our antigen prepared from blood-free microfilariae of *D. immitis* concentrated while alive and then lyophilized, also caused a precipitate to form in the sera of patients and dogs with filariasis (10).

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The authors wish to record appreciation of the cooperation of Commodore T. M. Rivers, MC (S) USNR, Medical Officer in Command, U. S. Naval Medical Research Unit No. 2.

Acknowledgement is made of the valuable assistance of K. F. Hanes, Chief Pharmacists' Mate, USNR, A. J. Barger, Pharmacists' Mate 1st Class, USNR, and Edward Fong, Pharmacists' Mate 1st Class, USNR.

The Bureau of Medicine and Surgery does not necessarily undertake to endorse views or opinions which may be expressed in this article.

Theoretically, microfilariae of *W. bancrofti* should give specific results. However, to secure a gram, dry weight, of such material it is calculated that it would require the processing of over one hundred liters of blood from individuals with average infections of 2,000 microfilariae per ml. The *D. immitis* material is more easily obtained and we were able to prepare sufficient quantities of an antigen freed of the formed elements and soluble substances of the blood.

It was decided that the *D. immitis* microfilarial antigen should be tested in an endemic area to obtain a definition of its immunological activity, account to be taken of such other host factors as, intestinal parasites, age, sex, elephantiasis, etc., that supposedly influence or alter the dermal reaction. The island of Okinawa afforded this opportunity during July-October 1945, and intradermal tests were conducted there with an antigen made from *D. immitis* microfilariae on a number of natives infected with *W. bancrofti*. Service personnel on Guam were tested as controls.

PREPARATION OF THE ANTIGENS

For details concerning the preparation of the microfilarial antigen to the point where the microfilariae are dried and stored, reference is made to the earlier report of Franks and Stoll (10). The microfilariae as well as the *D. immitis* employed in this study were obtained from dogs in New Jersey, U. S. A. *Ascaris lumbricoides* were collected at necropsies at the Agana Civilian Hospital, Guam. Only adult females of both *D. immitis* and *A. lumbricoides* were used. These worms were removed from their hosts with sterile precautions and were washed separately in numerous changes of sterile physiological saline solution until the wash water was negative when cultured. They were then suspended in a small amount of distilled water for a few moments to rinse away the saline, after which they were transferred to a flask and lyophilized. To spot false positive reaction which might be caused by host protein present in the alimentary tracts of the adult worms and microfilariae of *D. immitis* used as antigens, serum from an uninfected dog was employed as a control. The serum was also lyophilized.

From this point on, the preparation of the four above-mentioned antigens was identical. The dried material was triturated in a TenBroeck grinder with powdered pyrex glass added as additional abrasive. The ground material was taken up at an initial dilution of 1:100 in physiological saline solution and was alternately frozen and thawed thrice in a period of 24 hours. The extraction was allowed to proceed for another 24 hours at room temperature with an occasional shaking. It was then centrifuged at 3000 RPM for 15 minutes. After the supernate was passed through a Seitz filter, cultural tests were made for sterility, and merthiolate to a concentration of 1-10,000 was added.

Trichina antigen (larval *Trichinella spiralis*) provided by the National Institute of Health and physiological saline solution containing a 1-10,000 concentration of merthiolate were employed as further controls. The nitrogen content of each antigen is shown in table 1.

LABORATORY PROCEDURES

The examination of fecal specimens for helminthic ova was by dilution egg counting (11) and by the AEX modification devised by Loughlin and Stoll

(12) of Telemann's acid-ether concentration method, the latter demonstrating the presence of small numbers of eggs. For protozoa, specimens were examined by saline smear, and, from all positives, slides stained with hematoxylin were prepared. Final diagnosis was made from the stained preparation. The results of all fecal examinations are based on one stool per person.

The degree of microfilaremia of the patients was determined by the Knott (13) technic adapted for counting, a simple procedure which had been earlier and successfully used by Franks and Stoll (10) for counting microfilariae in dog blood. One milliliter of blood, accurately measured with a 1.0 ml. tuberculin syringe, was put in 4.0 ml. of 2 per cent formalin; the average of the number of microfilariae counted in three 0.1 ml. aliquots drawn after thorough mixture of the suspension, multiplied by the dilution factor, in this instance 50, provided a quantitative statement of the degree of filaremia, expressed in microfilariae per ml. of blood. For individuals with high counts, dilutions of 1-100 and 1-200 were used, and, whenever the count was less than 600 per ml. or apparently negative, the whole original Knott suspension was centrifuged for 10 minutes at 2000

TABLE 1
Nitrogen Content of the Antigens

ANTIGEN	MG. NITROGEN* PER 100 ML. OF 1.0 PER CENT SOLUTION
Microfilariae of <i>D. immitis</i>	3.0
Adult females of <i>D. immitis</i>	3.2
Adult female (<i>Ascaris lumbricoides</i>).....	3.0
Dog serum (control).....	10.5
Larvae of <i>Trichinella spiralis</i>	7.7

* Determinations of Lieutenant O. F. Binkley, H (S), USNR, U. S. Naval Medical Research Unit No. 2.

RPM and the microfilariae in the sediment were counted. All patients examined for microfilariae were bled between the hours of 2000 and 2200.

PATIENTS AND METHODS OF STUDY

Skin tests were done on over 400 patients. These patients fall into two natural groups. Group I was composed of service personnel. Sixty of these men, mostly from northern states, were stationed on Guam, in their first tour of duty outside of the United States, and were known not to have been in areas where filariasis is endemic. Fecal examination showed that 7 were lightly infected with intestinal helminths, and 53 were negative (table 5). They thus represented an admirable control group for evaluation of the test antigen. In addition to the above mentioned 60 non-filariated service personnel, there are classified in Group I, 9 patients (table 7) with a clinical diagnosis of filariasis, who were skin tested. Group II was composed of natives of an endemic area, Okinawa.

A discussion of the epidemiology of wuchereriosis on Okinawa is beyond the scope of this report; however, it is well to note that this subtropical island is provided with all the links in the chain necessary to assure the continued propagation

of the disease. The plains that everywhere are converted into rice paddies are breeding areas par excellence for mosquitoes, the *Aedes* and *Culex* mosquitoes present in large numbers have been demonstrated to be effective vectors, and the natives have a degree of filariation that provides an adequate seed-bed.

All the data incorporated in our analysis were collected from cases conforming to the following schedule: In the patients in Group II a night specimen of blood was drawn for examination for microfilariae and at the same time a history was taken relevant to their exposure to and symptoms of filariasis and other parasitic infections. Within the next few days, the patients were contacted again, sera were drawn for serological studies, the patients were given a physical examination, and skin tests were made on them. Labeled containers for fecal samples were provided and most of the specimens were returned to the laboratory within 24-36 hours. Fecal specimens were obtained on approximately 70 per cent of the persons receiving a skin test. Group I was processed in a similar manner except that examinations for microfilariae were done only on those persons who had been in an endemic area.

The intradermal tests were carried out by the injection into the volar surface of the forearm of approximately 0.025 ml. of antigenic material; this gave an initial wheal of 3-4 mm in diameter. The reactions were of an immediate type and usually reached their height in 12-15 minutes. A reaction was considered positive when the wheal caused by an antigen had a diameter twice that of the wheal caused by the physiological saline control. As a rule the positive reactions had a diameter 3-4 mm. greater than that of controls. Right angle diameters of wheals were measured, and, in most instances, tracings of the reactions were made. Depending on the size of the wheal and the presence or absence of pseudopodia, the reactions were interpreted as negative, slight, moderate or marked. Examples of various skin responses in subjects with filariasis are illustrated in figures 1-3. In some of the highly sensitive persons, and when concentrated extracts of the antigens were used, the injected areas became somewhat indurated and edematous, remaining so at times for from twelve to twenty-four or even forty-eight hours. Erythema, too, was often marked, but, in view of the fact that in most cases it was ill defined, it was not considered in the evaluation of reactions.

The Group II patients studied were evacuees from all sections of Okinawa, so that the series is fairly representative of the island's population. In the examination of 677 natives, selected at random, for circulating microfilariae, 26.6 per cent were found positive. Of 259 natives with this incidence of microfilaremia, 57 per cent had physical signs tenable with the diagnosis of filariasis. The incidence of filarial infection as shown by combined blood and clinical studies was 65 per cent. In table 2 is shown the incidence of physical signs of filariasis in both sexes and the relation to microfilaremia.

Table 3 illustrates the increased incidence of physical signs in people as they grow older. This is significant showing as it does a consistent relationship between the appearance of pathological changes and length of exposure to infection. Further reference will be made to this factor in the interpretation of dermal reactions.

As the result of diagnostic conservatism, the incidence of physical signs in our series may be too low, as questionable cases were placed in the normal group. Incipient stages of the disease were not encountered. Diagnosis was made on the characteristic involvement of lymph nodes, lymph channels, the extremities, and the scrotum. A remarkable feature of the data is the low incidence of elephantiasis. In our series there were only eight cases, a little more than 1.0 per cent; and the involvement in four patients was so slight as to be of no func-

TABLE 2

Incidence of Filarial Infection as Determined by Microfilaremia and by Physical Signs, in Relation to Sex

	MALES		FEMALES		TOTAL	
	Number examined	Per cent positive	Number examined	Per cent positive	Number examined	Per cent positive
A. Presence of microfilaremia.....	187	35.0	490	23.4	677	26.6
B* Presence of physical signs.....						
1) In 69 individuals with microfilaremia.....	26	84	43	57	69	67
2) In 190 individuals without microfilaremia.....	33	65	159	50	190	53

* In "B" a fair sample of 259 only of the 677 persons examined for microfilariae in "A" are tabulated.

TABLE 3

Incidence of Filarial Infection as Determined by Microfilaremia and by Physical Signs, in Relation to Age

AGE	NUMBER	PER CENT WITH MICRO-FILAREMIA	PER CENT WITH PHYSICAL SIGNS OF*	
			83 with microfilaremia	106 without microfilaremia
6-15	66	19	38	46
16-25	173	26	39	47
26-45	237	21	64	56
46-74	201	34	95	73
Total.....	677	26.6	67	53

* Of the 677 persons examined for microfilaremia, it will be noted that a fair sample of 194 only are classified for the presence of physical signs in relation to age.

tional handicap. Twenty other patients with elephantiasis were seen and in only one instance was multiple elephantiasis encountered.

It seems to us that this finding needs to be taken more generally into account in gauging the degree of filariation in an area. The usual assumption that a high incidence of filarial infection and elephantiasis go hand in hand was decidedly not a feature of filariasis on Okinawa. Here the demonstrated microfilarial carriers at 26.6 per cent approached the 30 per cent level which Napier (14) uses

as an index of a hyperendemic area. Combined blood and clinical studies indicated that the demonstrable incidence of filariasis in Okinawa was of the magnitude of 65 per cent, and was probably considerably higher.

Most cases that came to necropsy exhibited lesions that were compatible with a filarial infection; however, in many instances the findings were limited to the lymph nodes and lymph channels. With such findings and cognizant of O'Connor's statement that "Examination (i.e. tissue examination) of apparently normal persons in endemic areas indicates practically all inhabitants are infected with a few or many *Wuchereria*" it is justifiable for the purpose of evaluating our antigens to consider that all Okinawans, except very young infants are infected.

No intact adult worms were recovered at necropsy, but the confirmation of the infecting organism as *Wuchereria bancrofti* was established by examining microfilariae on stained smears. Four patients were bled every two to three hours for 24 hours, and the microfilariae were found to have a nocturnal type of periodicity, with the height of the microfilaremia coming between 2100 and 2400. The blood from all our cases was drawn between 2000 and 2200, and, in 180 positive cases, the counts varied from 1 to 23,000 microfilariae per ml.; 80 per cent of the cases had a thousand or less per ml., while only 6.0 per cent of individuals had counts of 4000 or more per ml. The percentage of positives for microfilariae increased significantly with age, especially when the children were contrasted with adults 46 years and older (table 3). The increased incidence of physical signs of filariasis in those individuals with microfilaremia, to which reference has already been made, was shown especially in the males (table 2) and in ages 26 and over (table 3). No positive correlation could be established between the numbers of circulating microfilariae and the age of the patients, nor with the degree of clinical filariasis. Four of the eight patients with elephantiasis had circulating microfilariae (only one had a high count) and three of these were among those who were mentioned previously as having minimal involvement.

RESULTS OF SKIN TESTS

Fifty-three service men of Group I, who had never been exposed to filariasis and who were not infected with intestinal helminths, were tested with various dilutions of the antigens to determine the optimal amounts for intradermal use. Thirty-nine of them did not react to any of the antigens in their strongest dilution, 1-1000. In table 4 is shown the greatest dilution of each antigen that provoked a reaction in 14 of these 53 Group I controls. None of the others reacted to any of the antigens.

As will be noted from data in table 4, three of the controls reacted to microfilarial antigen. Two of these (D. R. G. and M. R. K.) reacted to all of the materials including the saline control and were found to be sensitive to the preservative used. In addition, D. R. G. gave a history of being allergic to dog dander, timothy grass, and several kinds of food. Ten of the thirteen controls, reacting positively to the *D. immitis* adult antigen, were screened out at a dilution of 1:8000 or more and two showed a reaction to the dog serum control in the same dilution as that employed for the *D. immitis* adult antigen. Six of the subjects

gave reaction to the dog serum control; two of these are the aforementioned allergic individuals and three others were screened out a dilution of 1:8000. Thus, in 53 control subjects there was one false positive reaction to the microfilarial antigen, eleven to the *D. immitis* adult antigen and four to the dog serum control at a dilution of 1:1000. The merthiolate sensitive individuals are not included.

These results indicate that the microfilarial antigen can be used at a dilution of 1:1000, and, as noted by Bozicevich and Hutter (5), a dilution of 1:8000 of the adult *D. immitis* and dog serum control antigens screened out all but an occasional false positive reaction. These two dilutions (1:1000 for microfilarial antigen and 1:8000 for adult worm antigen) were accordingly utilized in our routine tests.

TABLE 4

*The Greatest Dilution of Microfilarial and Other Antigens Giving a Positive Intradermal Test in Service Personnel in Whom no Intestinal Helminthic Infection was Detected**

SUBJECTS	D. IMMITIS, MICROFILARIAL ANTIGEN	D. IMMITIS, ADULT ANTIGEN	DOG SERUM CONTROL	SALINE CONTROL
B. E. B.....	0	1:1000	0	0
D. A. B.....	1:1000	1:4000	0	0
J. R. C.....	0	1:4000	0	0
C. F. D.....	0	1:8000	1:1000	0
C. R. E.....	0	1:1000	1:1000	0
D. R. G.....	1:4000	1:16000	1:16000	Pos.
W. B. H.....	0	1:8000	1:8000	0
T. P. H.....	0	1:1000	0	0
M. R. K.....	Pos.	Pos.	Pos.	Pos.
T. E. K.....	0	1:1000	0	0
J. P. K.....	0	1:4000	0	0
R. I. R.....	0	1:1000	0	0
J. R.....	0	1:4000	0	0
C. E. W.....	0	0	1:1000	0

* The 39 persons who did not react to any of the antigens are not included in this table, nor is the record included of the reactions to 1/8000 dilution of adult *Ascaris* antigen. There were 8 of the 53 persons with no current helminthic infection who so reacted.

In addition, *Trichinella spiralis* antigen in a dilution of 1:8000 and saline controls, one with a preservative of phenol, the preservative used in the trichina antigen, and the other with merthiolate, were employed in certain experiments.

In table 5 are shown the results of intradermal tests on 7 additional service men from Group I who were shown to be infected with *Ascaris* or hookworm. Neither the microfilarial nor the *D. immitis* adult antigen reacted in these patients; however, it is of interest that the *Ascaris* antigen (1:8000) employed gave marked dermal responses in four of the five subjects with *Ascaris* infections. We noted that in our control group this dilution of *Ascaris* antigen gave 15 per cent false positive responses, but none of the reactions in them was so strongly positive as in the patients with *Ascaris*.

From Group II, altogether 341 Okinawans were tested with the microfilarial

TABLE 5

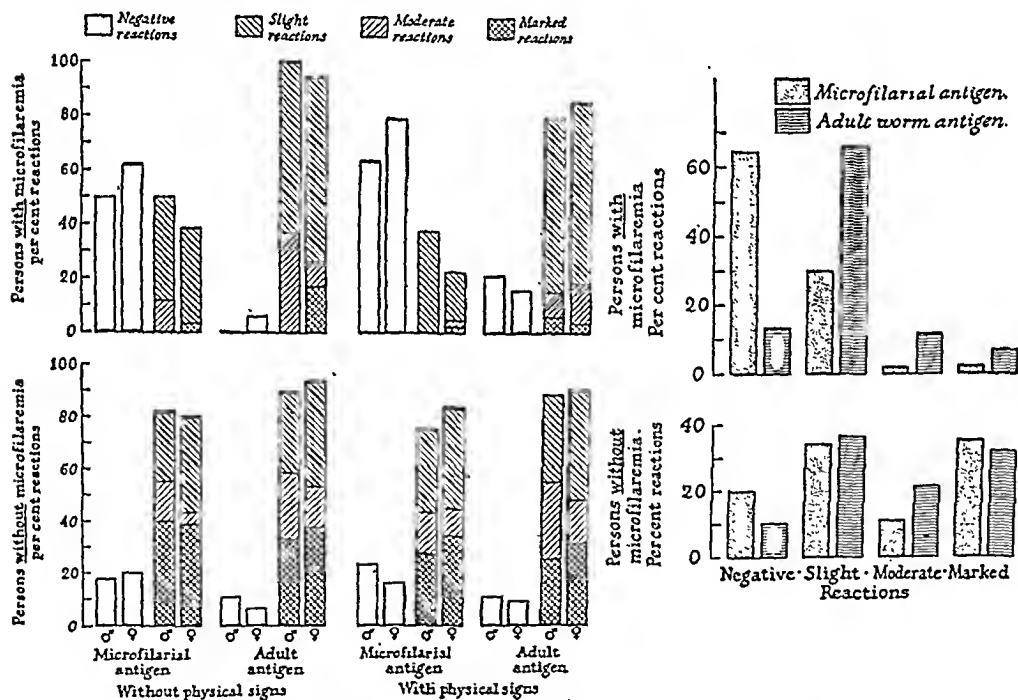
Results of Intradermal Tests with Microfilarial and Other Antigens in Service Personnel Infected with *Ascaris* and Hookworm

PATIENTS	DIAGNOSIS	DEGREE OF INFECTION	D. IMMITIS, MICRO-FILARIAL ANTIGEN, 1:1000	D. IMMITIS, ADULT ANTIGEN, 1:8000	ASCARIS ANTIGEN 1:8000	TRICHINA ANTIGEN 1:8000	DOG SERUM CONTROL 1:1000	SALINE CONTROL
F. T.....	<i>Ascaris</i>	Light	0	0	7X7*	0	0	0
J. H. M.....	Virgin	Light	0	0	15X8*	0	0	0
	<i>Ascaris</i>							
H. H. D.....	<i>Ascaris</i>	Light	0	0	7X7*	0	0	0
W. R. F.....	<i>Ascaris</i>	Heavy	0	0	11X10	0	0	0
C. H. A.....	<i>Ascaris</i>	Minimal	0	0	6X5	0	0	0
G. D. G.....	Male	One adult worm†	0	0	0	0	0	0
	<i>Necator</i>							
M. L. B.....	<i>Necator americanus</i>	Moderate	0	0	5X6	0	0	0

Measurements in mm.

* Pseudopodia present.

† Recovered by worm count after treatment.



GRAPH IA

GRAPH IIB

GRAPH IA. Results of intradermal tests with antigens of *Dirofilaria immitis* on natives of Okinawa. Microfilarial antigen was used at 1:1000 dilution, adult worm antigen at 1:8000 dilution. The bar diagrams are arranged to show the per cent of reactions among persons with and without microfilaremia (See also Graph IB), contrasting those with and without physical signs of filariasis. General similarity in the reactions of males and females will be noted. The results are based on 130 persons with microfilaremia of whom 88 showed physical signs, and on 190 persons without microfilaremia of whom 106 showed physical signs.

GRAPH IB. Composite results of intradermal tests on 320 natives of Okinawa with microfilarial and adult worm antigens of *D. immitis*. The data used are the same as in Graph IA but are here arranged to contrast the degree of skin reactions in the 130 persons with microfilaremia and 190 with no microfilaremia, without reference to physical signs of filariasis or to sex.

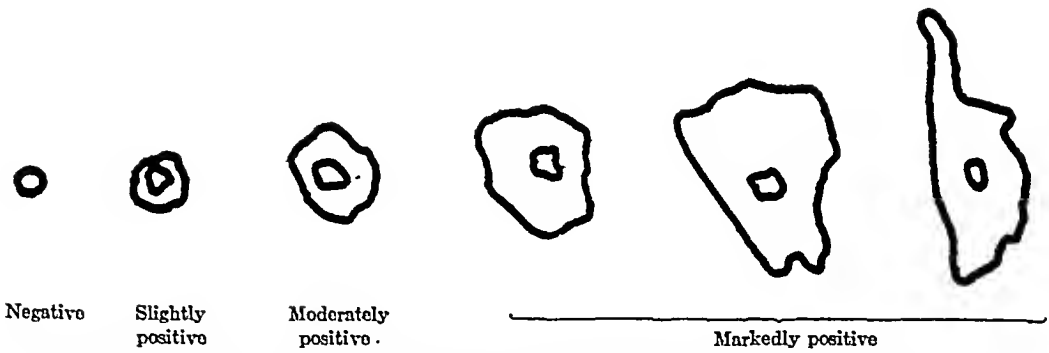


FIG. 1. Tracings, reproduced actual size, of examples of the various skin responses in persons infected with filariasis, made 10-15 minutes after injection of the test antigen. The inner circle represents the outline of the initial bleb.

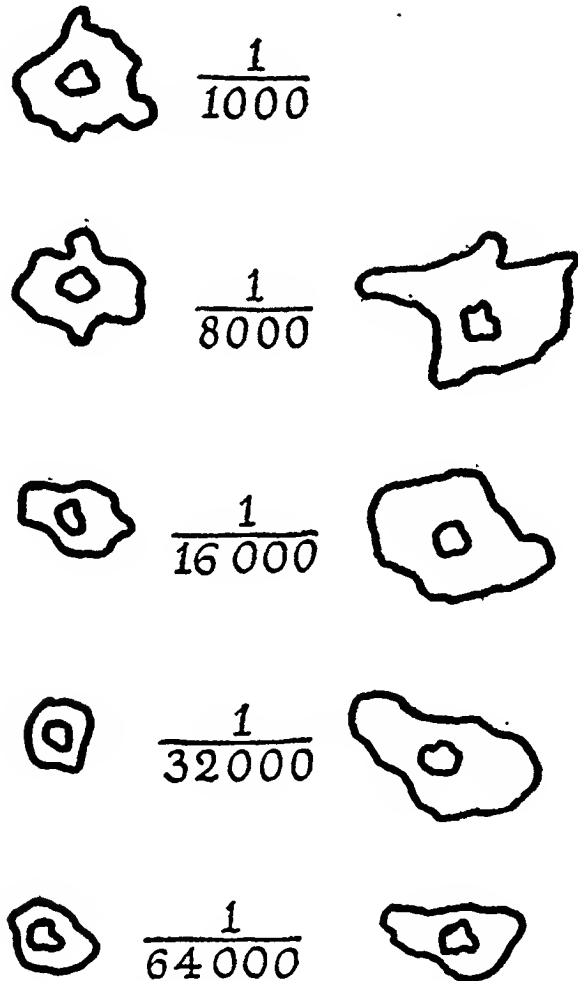


FIG. 2. Tracings, reproduced actual size, of the titration of microfilarial (on the left) and adult worm antigen (on the right) of *D. immitis* in skin tests on one patient with filariasis bancrofti. The inner circle represents the outline of the initial bleb.

antigen; however, our analysis is confined to the [320] subjects on which our studies were complete. The data are interesting from several angles and are best presented by a breakdown into two sub-groups, namely, natives with and natives without microfilaremia (*i.e.* demonstrated circulating microfilariae). A further division of the sub-groups according to sex and the presence of physical signs of filariasis is also given. Graphs IA and IB summarize the results of the intra-dermal tests with the microfilarial and adult worm antigens of *D. immitis* in these sub-groups. The responses are arranged to show their distribution according to the degree of reactivity as negative, slightly, moderately and markedly positive. Traced examples of these gradations of reactions and the results of the titration of the microfilarial and adult *D. immitis* antigens on one of the patients are shown in figures 1-2.

The incidence of negative reactions in the 320 natives, without reference to age, presence of microfilariae or physical signs, was 39 per cent for the microfilarial antigen and 11 per cent for the adult *D. immitis* antigen. When we

TABLE 6

Incidence of Reactions to D. immitis Microfilarial and Adult Antigen in Relation to Age in Subjects Without Microfilaremia

AGE	NUMBER	PER CENT OF NEGATIVES		PER CENT OF MARKEDLY POSITIVES	
		Microfilarial antigen	Adult worm antigen	Microfilarial antigen	Adult worm antigen
6-15	43	9	5	49	51
16-25	53	17	6	47	42
26-45	48	15	6	27	25
46-74	46	39	27	17	17

analyzed the dermal reactions of the 130 individuals with circulating microfilariae, we found that 67 per cent of them did not react to the microfilarial antigen, 30 per cent gave a slight reaction and only 3 per cent (four patients) gave a moderate to marked reaction. In the natives without a microfilaremia, 20 per cent gave no reaction, 34 per cent gave a slight dermal response, but 46 per cent gave moderate to marked reactions. The wheals encountered in the slight dermal responses measured approximately 6-8 mm. in diameter, while the markedly positive reactions varied from 12-25 mm. in diameter, all but a few having pseudopodia.

As will be noted in Graphs IA and IB, the incidences of negative reactions to the adult worm antigen are not significantly different in patients with and without circulating microfilariae; however, again most of the positive reactions in the group with microfilaremia are slight. In those without circulating microfilariae, the reactions to the *D. immitis* adult antigen parallel those to the microfilarial antigen. One of the patients with elephantiasis reacted to the microfilarial antigen and four gave slight reactions to the *D. immitis* adult antigen. It is notable that those who reacted were the patients with minimal involvement. Another point of significance was the increased incidence of negative reactions in older

age groups; conversely, of course, there were more positive ones in the younger patients. In table 6 is shown the incidence, according to age, of negative and markedly positive reactions to the microfilarial and the adult worm antigens in the group of subjects without microfilaremia. This general correlation holds also for the group of subjects with circulating microfilariae.

Of the natives tested, all gave negative responses to the dog serum and saline controls; 10 per cent (33 natives) were positive to the *Trichinella spiralis* antigen at a dilution of 1:8000. However, Fink's study of necropsy material on Okinawa did not demonstrate the presence of trichina infections (15). Only two of the

TABLE 7

Results of Intradermal Tests with Microfilarial and Other Antigens in Service Personnel with a Clinical Diagnosis of Filariasis

PATIENT	CLINICAL DIAGNOSIS	PLACE AND YEAR OF EXPOSURE	INTESTINAL PARASITES	MICROFILARIAL ANTIGEN 1:1000	D. IMMITIS ADULT ANTIGEN 1:8000	DOG SERUM CONTROL 1:1000	SALINE CONTROL
F. J. C....	Filariasis	Samoa, 1912	Hw; Trich.	11X11*	9X9	0	0
W. L. C....	Filariasis	Samoa, 1912	Hw; Trich.	12X15*	10X10	0	0
D. F. C....	Filariasis	Samoa, 1913	<i>Endolimax nana</i>	7X8	5X5	0	0
A. E. H....	Filariasis	Samoa, 1912	Trich.	8X14*	6X10	0	0
J. E. G....	Filariasis	Samoa, 1912	Negative	15X8*	11X12*	3X3	0
A. W. C....	Filariasis	Okinawa, 1945	<i>Hymenolepis nana</i>	0	6X8	0	0
E. A. B....	Filariasis	Okinawa, 1945	Hw; Trich., <i>S. japonicum</i>	0	8X6	0	0
J. D. S....	?Filariasis	Guadalcanal, 1943	<i>E. histolytica</i>	6X7	8X7	6X7	0
W. F. B....	?Filariasis	Guadalcanal, 1943	Negative	0	0	0	0

Measurements in mm.

* Pseudopodia present.

Hw = hookworm; Trich. = *Trichuris trichiura*.

33 patients tested gave strongly positive reactions and in only one of them was the response as marked as the reaction to the filarial antigen. In the presence of a filarial infection and in the absence of clinical and laboratory evidence of *Trichinella spiralis* infection, it is unlikely that the reaction to the trichina antigen was specific.

The possibility remains open that unaltered rat protein in the alimentary tracts of the trichina larvae routinely used in the making of U.S.P.H.S. trichina antigen (16) may be the cause of this positive reaction in native populations, rather than trichina infection itself.

Opportunity was not afforded to make a comparable study in service personnel, as the number of troops with filariasis available for testing was limited. However, complete studies were carried out on nine patients in the wards of U. S. Naval Medical Research Unit No. 2. In several of these, the diagnosis had been

established on the basis of a history of exposure and the usual signs of lymphangitis, lymphadenopathy, involvement of scrotum, etc. In none of the patients

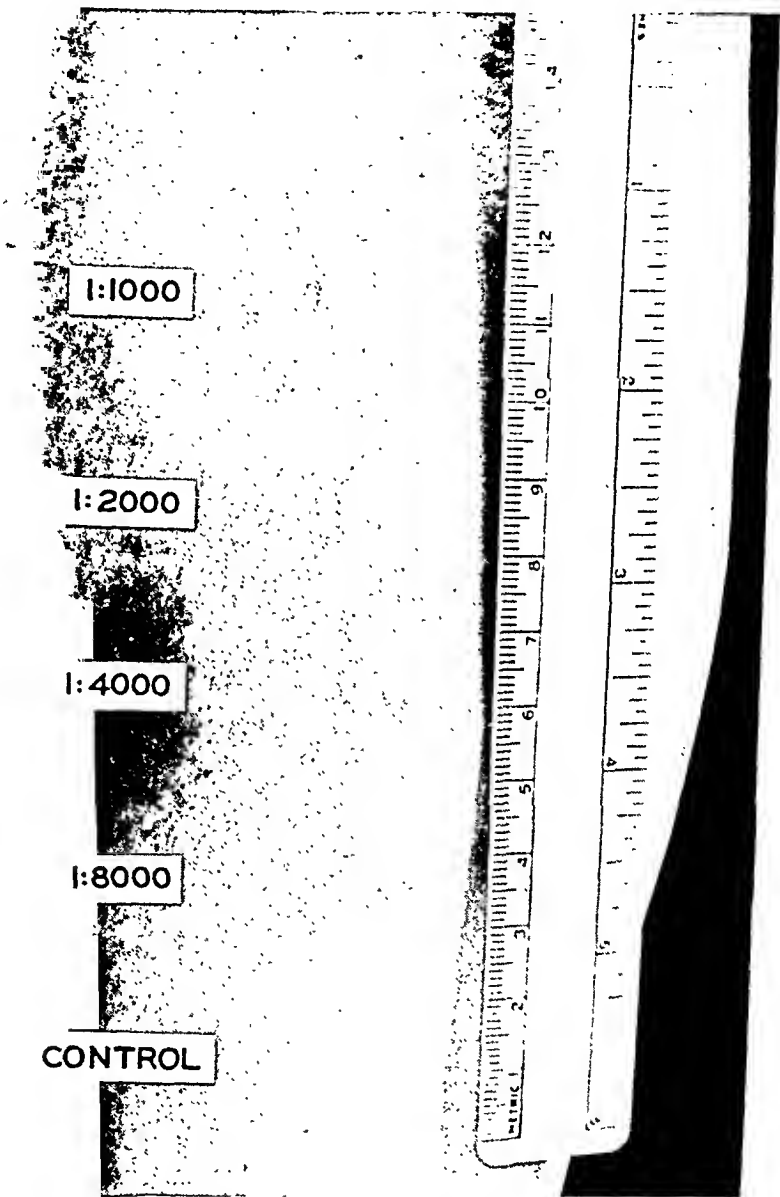


FIG. 3. Photograph of reactions obtained in a patient with filariasis 15 minutes after the intradermal injection of various dilutions of the *D. immitis* microfilarial antigen.

had microfilariae been found. The results of tests on these men are summarized in table 7.

The five patients from Samoa, who had had filariasis for three years or more, gave intradermal tests that were of definite value in confirming the clinical diagnosis. The intradermal reactions of a marine (J. E. G.), infected with filariasis,

to various dilutions of the microfilarial antigen is shown in figure 3. In the patients from Guadalcanal, the diagnosis had not been established clinically. One of them gave a history of two bouts of lymphangitis while on Guadalcanal in 1943, and on examination he was found to have a moderate, generalized lymphadenopathy. His dermal reactions were negative. The other man, who had no signs or symptoms of filariasis, had been on Guadalcanal for a year. His skin test was positive, but he reacted to the dog serum control in the same dilution as that employed for the microfilarial antigen; this would be classed as an unsatisfactory test. It is questionable whether or not Guadalcanal was a source of filarial infection for our troops, and it is unlikely that these two patients had filariasis. The two soldiers infected in Okinawa unquestionably had early clinical filariasis. They had had their infection for only 4-6 months and reacted to the adult *D. immitis* antigen but not to the microfilarial antigen. The significance of these reactions will be discussed later.

TABLE 8

Severity of Infection with Hookworm, Ascaris and Trichuris as Determined by Egg Counts In Contrasting 58 Individuals age 6-15 with 144 Individuals age 16-74

INTENSITY OF INFECTION	PER CENT FOR HOOKWORM		PER CENT FOR ASCARIS		PER CENT FOR TRICHURIS	
	Age group					
	6-15	16-74	6-15	16-74	6-15	16-74
Negative.....	21	18	42	54	75	90
Minimal.....	52	42	10	12	24	10
Moderate.....	25	38	42	32	1	0
Heavy.....	2	2	6	2	0	0

The lack of specificity when simple extracts of adult nematodes are used as test antigens in individuals parasitized by heterologous nematodes has generally been recognized. Several investigators working with filarids as a test antigen have ascribed their false positive reactions to a possible crossing with intestinal helminths. Okinawa provided an opportunity to study the skin responses of 202 natives on whom helminthic studies had been made.

From results shown in table 8, it is apparent that the incidence of intestinal helminthiasis was high in the Okinawan natives involved in this study, but that severity of infection, as determined by egg counts, was not great. This fact was verified at the necropsy table where the average collection was only 10 worms per patient in one series of 35 infected with hookworm. *Ascaris lumbricoides* was not found so frequently as was hookworm, predominantly *Necator americanus* (17), and heavy infections made up only a small portion of the total, even in children. The incidence and severity of *Trichuris trichiura* infections were low.

No significant correlation was found when the dermal response of the Okinawan native was examined in relation to his intestinal helminthic fauna, whether with reference to the helminthic species involved, the severity of the individual infection,

tion, or the age of the individual. In fact, the heaviest *Ascaris* infections encountered were in the age group 6-15 which gave the poorest results with the filarial antigens. If a crossing is expected, it would appear to have been in this group that it should have occurred.

Twenty-three per cent of the natives were infected with *E. histolytica* and altogether 49 per cent showed infections with other intestinal protozoa. No non-specific reactions to the filarial antigens as the result of protozoan infection could be demonstrated.

DISCUSSION

In our study we found, as others have found for antigens prepared from adult filarids, that an antigen made from microfilariae of *Dirofilaria immitis*, the dog heart worm, has a limited usefulness as a diagnostic aid in filariasis. It is more specific in the sense that fewer false positive reactions are elicited by it in non-filarial persons than are provoked by antigens made from adult female *D. immitis*. For this reason it may be used at stronger concentrations, and in this report was employed at 1:1000 as compared to 1:8000 for adult worm antigen.

The reactivity of the test antigen in a filarial patient is apparently influenced by the clinical stage of the disease. In 320 natives of a filarial area like Okinawa, only 61 per cent gave positive responses to the microfilarial antigen. The remarkable feature of the data is that most of the negative reactions were given by that group of persons having circulating microfilariae. In contrast to the 67 per cent of negative reactions in the group mentioned in the preceding sentence, only 20 per cent of the natives without microfilariae gave negative dermal tests. The degree of microfilaremia is not a factor, as a negative response occurred as often in natives with low counts as in those with hyperfilaremia. It is significant, too, that when positive dermal responses were elicited in the group with microfilariae, they were slight.

More negative skin reactions were obtained in the older age groups. This relationship was observed in natives without microfilaremia, so that the presence of microfilariae was not a factor in the incidence of negatives in this correlation. The Okinawans whom we studied gave the impression of being a homogeneous population in which the variables influencing their exposure to filariasis were slight. The age resistance demonstrated is not merely an extension of the natural immunity of the host, but is a measure of the immunity that individuals develop in relation to the length of their exposure to, and degree of, infection. Reference is made again to the incidence of clinical filariasis in the various age groups; the parallelism between these two correlations is evident. The negative reactions obtained in practically all the patients with elephantiasis is the most striking illustration of this relationship.

The two Group I patients (servicemen) with recently acquired filariasis gave negative dermal responses to the microfilarial antigen. This suggests that individuals infected for so short a time as 4-6 months have not yet produced much antibody related to the larval stage of the filarid. A mild dermal reaction, however, was provoked with adult worm antigen. Presumably, it is only antibody related to the developing or adult stages of the worm which is being produced in

this early phase of the disease. In the service personnel (table 7) with infections of 2 to 3 years duration, antibodies to both antigens were demonstrated.

The service personnel and many of the natives with intestinal helminthiasis did not react to the filarial antigens. In the patients of our series, the infections for the most part were light and it is possible that in a heavily parasitized population an appreciable number of false positive reactions would have been elicited.

The occurrence of reactions to *Trichinella spiralis* antigen in a filarial population where no evidence of trichina infection exists is of interest. Possibly the antigenic stimulation of a somatic parasite is sufficiently effective that non-specific reactions to the microfilarial antigen are more likely to occur in its presence than in infections with intestinal nematodes. Unfortunately no opportunity was provided to try the filarial antigens on persons known to be infected with *Trichinella spiralis*. In testing for filariasis in a population whose nematode fauna is not known, it thus becomes important to employ a control of trichina antigen as well as to evaluate the group's intestinal helminthic infections. The possibility has already been suggested that the reaction to trichina antigen may in part be due to the presence of rat protein associated with an antigen derived from infected rats (16). Further definition of the trichina reactivity could be secured by using trichina antigen derived from rabbit, guinea pig or swine hosts to supplement the information obtained by trichina antigen derived from rats.

A preliminary definition of the limitations and the advantages of the microfilarial antigen can now be drawn. When compared on a basis of dry weight of lyophilized antigen, the microfilarial antigen gives 90 per cent fewer false positive reactions in normal controls and in patients with intestinal helminthiasis than does the antigen prepared from adult female *D. immitis*. In contrast to the adult worm antigen (at 1:8000) the microfilarial antigen can be employed at an optimal dilution (1:1000) which will give reactions that are of greater significance. A negative dermal response to the microfilarial antigen is often encountered under the following circumstances: in the early and acute stages of filariasis, possibly up to a year following the initial exposure; in persons with circulating microfilariac, irrespective of the degree of microfilaremia; and, in persons whose exposure has been long and who have developed many of the clinical signs of advanced filariasis. Light infections of intestinal helminths evidently do not influence the reaction at the dilution of the antigen employed. However, additional work is needed to evaluate the nonspecific reactions in persons heavily parasitized, either with intestinal or somatic parasites.

It is well to emphasize the fact that the microfilarial antigen has its optimal usefulness in patients whose degree of filariation is slight to the extent that they are not desensitized by antigen arising from their own infection. It has been in this group, of which the infected service personnel is representative, that the diagnosis has been the greatest problem. Despite the limitations outlined, the reactions in this group were satisfactory and acted as an aid in confirming the clinical diagnosis of filariasis.

In experiments as yet incomplete, a circulating antigen has been demonstrated which provides a partial explanation for the pattern of responses noted. The findings are discussed in another report (18).

It is evident that no single antigen has the range of activity that will provide a confirmatory test in each individual case of filariasis. The varied pattern of the responses in this disease indicates that a better understanding of the immunological principles involved is necessary, if we are to utilize intelligently the diagnostic tests available, limited as they are.

SUMMARY

Over 400 patients were tested with an antigen prepared from the microfilariae of *D. immitis*. The optimal dilution for testing was determined, and the varied responses in different stages of filariasis were demonstrated. Its activity in comparison to that of the adult worm antigen was shown, and its limitation and advantages were discussed. The role of intestinal and somatic helminths in eliciting nonspecific reactions was appraised.

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EVALUATION OF PENICILLIN IN THE TREATMENT OF YAWS

FINAL REPORT¹

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In a preliminary paper (1) the immediate results of treatment of 500 patients with primary and secondary yaws (frambæsia) infections in Haiti with penicillin were reported. Clinical and serologic follow-up observations over a period of six months on the first 200 patients treated with penicillin in aqueous solution, and over a three months' period on the remaining patients treated with penicillin in oil with beeswax, were included in that report. The present paper constitutes a final report of up to 12 months follow-up observations on the entire group of patients.

The patients were divided into three series as follows:

Series A. Two hundred patients were hospitalized and given a total of 1,200,000 units of penicillin sodium in aqueous solution each over a period of four days. They received 30 intramuscular injections of 40,000 units each, one every three hours day and night. All patients received the same total dose, regardless of age. The age distribution of the patients is given in table 1.

Series B. One hundred and fifty-one patients were treated on a two-day ambulatory schedule with penicillin calcium in peanut oil with 4.8 per cent beeswax by weight (300,000 units per cc.). The dosage was graded down for children on the basis of age and approximate weight. The age distribution of the patients and the total amounts of penicillin administered are given in table 1. Children 6 to 12 years of age received 600,000 units; patients from 13 to 16 years of age, 900,000 units; and those 17 years and over, 1,200,000 units. The drug was given by intramuscular injection in divided doses 24 hours apart.

Series C. One hundred and forty-nine patients were treated on a one-day ambulatory schedule with penicillin calcium in oil with beeswax. The dosage was graded down for children as in series B. The age distribution of the patients and the total amounts of penicillin administered are given in table 1. The drug was given by intramuscular injection in divided doses 10 to 12 hours apart.

A medical history was taken and blood for serologic testing was collected from each patient prior to treatment. Physical examination was limited to close observation of the skin and muco-cutaneous borders. Blood serum was placed in

¹ Cooperative research project of the Division of Health and Sanitation, Office of Inter-American Affairs; Preventive Medicine Service, Office of The Surgeon General, U. S. Army; Divisions of Serology and Parasitology, Army Medical School, Army Medical Center; and the Government of the Republic of Haiti. The writers are indebted to Dr. J. E. Moore, Johns Hopkins Hospital, for aid in preparation of the manuscript.

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vials containing powdered merthiolate, 1 mg. per cc. of serum (2), and shipped by air to the Division of Serology, Army Medical School, Army Medical Center, Washington, D. C. Less than 1 per cent of the specimens arrived there unsatisfactory for testing as the result of bacterial contamination. A battery of serologic tests was performed on each specimen: Kline diagnostic, Kline exclusion, a new microflocculation test employing cardiolipin antigen (3), Mazzini, quantitative Kahn, and quantitative Kolmer. Only the quantitative Kahn titers are reported in this paper. The other tests employed in the sero-diagnostic battery were more sensitive than the Kahn and gave higher titered reactions, but the general pattern and the amount of serologic improvement was similar in all tests.

TABLE 1

Age Distribution of Patients with Primary and Secondary Yaws Infections Treated with Penicillin in Aqueous Solution and in Oil with Beeswax

AGE GROUPS	5 years and under	6-12 years	13-16 years	17 years and over	Total
Series A					
Number of patients.....	13	60	41	86	200
Total dosage penicillin in aqueous solution (units).....	1,200,000	1,200,000	1,200,000	1,200,000	
Series B					
Number of patients.....	0	74	27	50	151
Total dosage penicillin in oil with beeswax (units).....		600,000	900,000	1,200,000	
Series C					
Number of patients.....	0	56	35	58	149
Total dosage penicillin in oil with beeswax (units).....		600,000	900,000	1,200,000	
Total number of patients.....	13	190	103	194	500

Follow-up clinical and serologic observations were made at monthly intervals on patients in series A and at three months' intervals on those in series B and C. In a number of instances blood specimens were lost, the tubes broken in shipment or the quantities of serum were insufficient for testing. Although a clinical examination had been made on these patients, that particular month's observation was not included in the present analysis since a corresponding serologic observation could not be made.

RESULTS OF TREATMENT

As reported previously (1) the clinical improvement of patients in series A during and immediately following treatment was rapid and remarkable. Joint pains disappeared in 24 to 48 hours; plantar and palmar "crab" lesions became painless in 48 to 72 hours. Both primary and secondary lesions began to dry up in 24 hours. Epithelium grew in from the periphery and completely covered

most lesions in three or four days. The great majority of patients who returned for observation one month after treatment showed complete healing of all lesions. A few ulcerated primary lesions with secondary bacterial infections were still draining pus at this time. Most of these healed spontaneously between the first and second month after treatment. Since patients in series B and C were treated on an ambulatory basis, it was not possible to follow their immediate clinical courses. However, when these patients were seen three months after treatment, their first follow-up observation, they showed complete healing of all lesions in most instances. Histologic studies were made on biopsy specimens taken before and after treatment on 10 patients in series A. The results are given in the preliminary report and will not be repeated here.

As previously reported (1) no severe toxic reactions were encountered. Approximately one-half of the patients in series A had a rise in temperature to 100°-104°F. two to eight hours after treatment was started. All temperatures gradually returned to normal in 10 to 12 hours. Approximately one-fifth of the patients showed a brief secondary elevation of temperature on the third, fourth, and fifth days of treatment. Temperatures were not taken on patients in series B and C. One patient in series B developed pain, heat, redness and swelling at the site of inoculation of penicillin in oil with beeswax in 24 hours.

With regard to serologic observations it was pointed out previously (1) that although the great majority of patients in series A showed a definite progressive reduction in Kahn titer during the first six months after treatment, only 10 per cent attained negativity at the end of this time. In general, the same held true for patients in series B and C three months after treatment. This and subsequent clinical and serologic observations over a 12 months' period made it necessary to set up certain arbitrary criteria upon which to evaluate the results of treatment. The following definitions were decided upon:

"Apparent cure" designates that during the period of observation the patient showed complete healing of primary and/or secondary skin lesions, together with reversal of the Kahn test to persistent negativity.

"Satisfactory progress" designates that during the period of observation the patient showed complete healing of primary and/or secondary skin lesions, together with progressive reduction of the Kahn titer to a persistent low level, but never attaining negativity.

"Unsatisfactory progress" designates that during the period of observation one or more of the following occurred: "reinfection", "clinical relapse", or "serologic relapse."

"Reinfection" indicates the development of a single new dark-field positive lesion with the clinical characteristics of primary yaws. In such patients the Kahn test was either positive at the time the new lesion was first observed or became positive thereafter. Its differentiation from "clinical relapse" is admittedly debatable.

"Clinical relapse" designates the development of new, multiple, dark-field positive secondary lesions, together with "serologic relapse", in the absence of a preceding primary lesion.

"Serologic relapse" indicates either the reversal of a previously negative Kahn test to persistent positivity, or a progressive increase in serologic titer following an initial decline, in the absence of clinical activity. To simplify presentation of data, patients whose Kahn titers failed to drop below the pre-treatment level are included in this category.

TABLE 2

Results of Treatment of Patients with Primary and Secondary Yaws in Series A with Penicillin in Aqueous Solution

(30,000 Units every 3 Hours to Total of 1,200,000 Units)

	PERIOD OF OBSERVATION (MONTHS)				TOTAL NUMBER OF PATIENTS	PER CENT OF TOTAL
	0-3	4-6	7-9	10-12		
"Apparent cure".....	0	0	5	47	52	26.7
"Satisfactory progress".....	5	5	7	106	123	63.5
"Apparent cure" plus "satisfactory progress".....	5	5	12	153	175	90.2
"Unsatisfactory progress".....	0	1	3	15	19	9.8
Total.....	5	6	15	168	194*	

* Six patients dropped from series because pre- or post-treatment serum was either unsatisfactory for testing or not obtained.

TABLE 3

Results of Treatment of Patients with Primary and Secondary Yaws in Series B with Penicillin in Oil with Beeswax

(1,200,000 Units in Divided Doses 24 Hours Apart)

	PERIOD OF OBSERVATION (MONTHS)				TOTAL NUMBER OF PATIENTS	PER CENT OF TOTAL
	3	6	9	12		
"Apparent cure".....	0	1	3	10	14	11.0
"Satisfactory progress".....	14	8	17	65	104	81.9
"Apparent cure" plus "satisfactory progress".....	14	9	20	75	118	92.9
"Unsatisfactory progress".....	0	1	5	3	9	7.1
Total.....	14	10	25	78	127*	

* Twenty-four patients dropped from series because pre- or post-treatment serum was either unsatisfactory for testing or not obtained.

The results of treatment of hospitalized patients in series A with multiple injections of penicillin in aqueous solution over a four-day period are given in table 2. It will be noted that 168 of 194 patients (84.5 per cent) were followed for a period of 10-12 months. Only 26.7 per cent of the entire group of patients could be adjudged as "apparent cures." However, an additional 63.5 per cent showed "satisfactory progress", totaling 90.2 per cent. The remaining 9.8 per cent of the patients were considered to show "unsatisfactory progress." From table 5,

it will be seen that 13 of the 19 patients in this category had "serologic relapses", the great majority of which occurred 10-12 months after treatment.

TABLE 4

Results of Treatment of Patients with Primary and Secondary Yaws in Series C with Penicillin in Oil with Beeswax

(1,200,000 Units in Divided Doses 10-12 Hours Apart)

	PERIOD OF OBSERVATION (MONTHS)				TOTAL NUMBER OF PATIENTS	PER CENT OF TOTAL
	3	6	9	12		
"Apparent cure".....	0	0	1	7	8	6.4
"Satisfactory progress".....	13	10	23	62	108	86.4
"Apparent Cure" plus "satisfactory progress".....	13	10	24	69	116	92.8
"Unsatisfactory progress".....	1	1	2	5	9	7.2
Total.....	14	11	26	74	125*	

* Twenty-four patients dropped from series because pre- or post-treatment serum was either unsatisfactory for testing or not obtained.

TABLE 5

Summary of Cases of Primary and Secondary Yaws Treated with Penicillin in Aqueous Solution (Series A) and Penicillin in Oil with Beeswax (Series B and C) which Showed "Unsatisfactory Progress"

	PERIOD OF OBSERVATION (MONTHS)				TOTAL NUMBER OF PATIENTS	PER CENT OF TOTAL
	0-3	4-6	7-9	10-12		
Series A						
"Reinfection".....	0	0	3	3	6	3.1
"Clinical relapse".....	0	0	0	0	0	0.0
"Serologic relapse".....	0	1	0	12	13	6.7
Total.....	0	1	3	15	19	9.8
Series B						
"Reinfection".....	1	0	0	1	2	1.6
"Clinical relapse".....	0	0	2	1	3	2.4
"Serologic relapse".....	0	1	2	1	4	3.1
Total.....	1	1	4	3	9	7.1
Series C						
"Reinfection".....	0	1	1	1	3	2.4
"Clinical relapse".....	0	1	0	0	1	0.8
"Serologic relapse".....	2	2	1	0	5	4.0
Total.....	2	4	2	1	9	7.2
Grand total.....	3	6	9	19	37	8.3

The results of treatment of ambulatory patients in series B with penicillin in oil with beeswax in divided doses 24 hours apart are given in table 3. Seventy-eight of 127 patients (61.5 per cent) were followed for a period of 12 months. Of

the entire group of patients, only 11.0 per cent could be designated as "apparent cures." An additional 81.9 per cent showed "satisfactory progress", totaling 92.9 per cent. The remaining 7.1 per cent were considered to show "unsatisfactory progress" (table 5).

The results of treatment of ambulatory patients in series C with penicillin in oil with beeswax in divided doses 10 to 12 hours apart are given in table 4. A smaller per cent of the patients, 59.2, were followed over a 12 months' period than in series A and B. Here, again, only a small per cent of the patients, 6.4, could be designated as "apparent cures." An additional 86.4 per cent of the patients showed "satisfactory progress", totaling 92.8 per cent. The remaining 7.2 per cent showed "unsatisfactory progress" (table 5).

DISCUSSION

The clinical response of primary and secondary yaws infections to penicillin therapy was uniformly excellent. The great majority of lesions healed completely within one week. A few secondarily infected lesions healed more slowly. On the other hand, the serologic response to treatment was strikingly different. Only 16.6 per cent of the entire group of 446 patients followed for varying lengths of time up to 12 months attained seronegativity and could be considered as "apparent cures" (table 6). However, in an additional 75.1 per cent of the patients there occurred a progressive reduction of the Kahn titer to a persistent low level ("satisfactory progress"). It is possible that if these patients could be observed over the period of another year or two, many of them would attain seronegativity. Such was the experience of Chambers (4) who reported that 25 per cent of 411 yaws patients treated with four to six weekly injections of neoarsphenamine developed negative Wassermann tests six months after treatment, 44 per cent 12 months after treatment, 59 per cent 18 months after treatment, and 68.4 per cent 24 months after treatment. He reported similar results on 143 yaws cases treated with four to six weekly injections of bismuth. It is of interest that a higher per cent of patients, 44, treated with neoarsphenamine attained seronegativity at the end of 12 months than those, 16.6, treated with penicillin. A strict comparison of these figures is not valid because the relative sensitivity of the two serodiagnostic tests employed is not known. Also, the latter figure is considerably lower than the per cent of serologic reversals obtained in patients with early active syphilis treated with a similar amount of penicillin (5). Aside from the fact that 12 months' observation of the yaws patients following penicillin therapy is obviously insufficient, no further explanation can be offered for the persistent positive serologic tests. It is possible that the almost constant exposure of these patients to reinfection may play an important role in this connection. From table 1, it will be seen that 73 of the patients in series A were children under 12 years of age. These patients received the same amount of penicillin as the adults, namely 1,200,000 units. It is significant that although the majority of these children received as much as five times more penicillin in proportion to body weight than adults, the serologic response was in no way different from that of the adults. This would seem to indicate that

the amount of penicillin beyond a certain minimum was not the essential factor in producing serologic reversals.

In table 6 is given a comparison of the results of treatment of the patients treated with penicillin in aqueous solution with those treated with penicillin in oil with beeswax. It is realized that this comparison is not strictly valid because of the difference in treatment schedules and because of the recent information (6) concerning the varying proportions of the four crystalline species of penicillin, G, F, X, and K, in commercial penicillins. Sternberg and Leifer (7) stated that in so far as can be learned, the change in commercial penicillins came in 1945. Patients in series A were treated in December 1944; patients in series B during March and April 1945; and patients in series C in May 1945.

TABLE 6

Comparison of Results of Treatment of Patients with Primary and Secondary Yaws with Penicillin in Aqueous Solution (Series A) and in Oil with Beeswax (Series B and C)

	SERIES A		SERIES B		SERIES C		TOTAL	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
"Apparent cure".....	52	26.7	14	11.0	8	6.4	74	16.6
"Satisfactory progress"....	123	63.5	104	81.9	108	86.4	335	75.1
"Apparent cure" plus "satisfactory progress"....	175	90.2	118	92.9	116	92.8	409	91.7
"Unsatisfactory progress"....	19	9.8	9	7.1	9	7.2	37	8.3
Dropped from series*.....	6		24		24		54	
Total.....	200		151		149		500	

* Cases in which pre- or post-treatment sera were either unsatisfactory for testing or not obtained.

The per cent of "apparent cures" was considerably higher in series A, 26.7, than in series B, 11.0, or series C, 6.4. It seems likely that this may have been due to the fact that the patients in series A were treated over a longer period of time, four days, than those in series B, two days, and series C, one day. However, in the latter two series the per cent of cases considered to show "satisfactory progress" was proportionately higher, 81.9 and 86.4, respectively, than in series A, 63.5. When one combines the two categories, "apparent cure" plus "satisfactory progress", the total per cent is almost identical for the three series: 90.2, 92.9, and 92.8, respectively. Thus, 91.7 per cent of all the patients treated fell into the category of "apparent cure" or "satisfactory progress." The remaining 8.3 per cent of the patients were considered to show "unsatisfactory progress." There was no appreciable difference in the per cent of cases in this category in the three series. From table 5, it will be noted the majority of these cases, 4.9 per cent, were termed "serologic relapse."

In view of the fact that the patients were almost constantly exposed, surprisingly few, 2.0 per cent, became reinfected. In only one case was the Kahn test observed to revert to negativity prior to the appearance of the supposed new

primary lesion. In approximately one-half of the cases of "reinfection" an increase in the Kahn titer was detected one or more months before the supposed primary lesion developed; in the remainder of the cases an increase in Kahn titer was noted simultaneous with or after the appearance of the lesion. With regard to "clinical relapses", the incidence of which was even lower than that of "reinfection", 0.9 per cent, in no instance did the Kahn test revert to negativity prior to the appearance of the supposed new secondary lesions. In each instance an increase in the Kahn titer was detected at the same time the lesions appeared.

A large number of the patients in this study had been treated previously with mapharsen and/or bismuth and subsequently relapsed or became reinfected. Their response to treatment with penicillin was similar to that of the previously untreated patients. The duration of infection in the patients apparently had no effect on their response to penicillin therapy.

SUMMARY

An analysis of the results of treatment of 500 cases of primary and secondary yaws infections with penicillin, in aqueous solution and in oil with beeswax, is presented. Follow-up clinical and serologic observations were made on 446 of these patients for varying lengths of time up to 12 months. Three hundred and twenty (71.7 per cent) were followed for a period of 10 to 12 months after treatment.

Clinical response to treatment was uniformly excellent, but serologic response was not. Only 16.6 per cent of the 446 patients were considered to show "apparent cure." An additional 75.1 per cent showed "satisfactory progress", totaling 91.7 per cent. The remaining 8.3 per cent of the patients showed "unsatisfactory progress." In the latter group were included cases of "reinfection", "clinical relapse", and "serologic relapse."

The per cent of "apparent cures" was higher, 26.7, in the group of hospitalized patients treated over a four-day period with penicillin in aqueous solution than in the two groups treated with penicillin in oil with beeswax on an ambulatory basis over two days, 11.0, and one day, 6.4. However, there was a correspondingly higher proportion of cases showing "satisfactory progress" in the latter two groups of patients so that when one combines cases of "apparent cure" with those showing "satisfactory progress", the total per cent is almost identical in the three groups of patients, 90.2, 92.9, and 92.8, respectively.

It is appreciated that follow-up observations over a period of 10 to 12 months after treatment are not sufficient to permit a comprehensive evaluation of the efficacy of penicillin in the treatment of yaws. Also, it is not possible to make a strict comparison of the results of treatment of penicillin in aqueous solution with penicillin in oil with beeswax, because of the difference in treatment schedules employed. However, it is felt that penicillin is probably the present-day drug of choice in the treatment of yaws, and that penicillin in oil with beeswax is of considerable public health value in countries such as Haiti where large numbers of patients must be treated on an ambulatory basis in rural clinics. Its use can be expected to successfully control cutaneous lesions and therefore prevent the spread of infection.

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SALMONELLA INFECTIONS IN PANAMA

REVIEW OF 219 CONSECUTIVE HOSPITAL CASES OCCURRING IN THE 5 YEAR PERIOD 1942-1946

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Only in the past decade has the importance of Salmonella infections in human pathology come to be recognized. Many studies have been made, the reports of which disclose the varied manifestations of the disease. Most of these have dealt with a particular phase of the problem, while relatively few have presented a complete overall picture. Among the latter are Bornstein's discussion (1) and the New York Salmonella Center reports (2, 3) which the authors believe to represent a fair sampling of the infection as it occurs in the United States.

The present study was undertaken with a similar purpose in mind. It is an attempt to present the various aspects of the infection as it occurs on the Isthmus of Panama.

MATERIAL

Since 1941 cultures of all organisms identified by the Gorgas Hospital Laboratory (Canal Zone Board of Health Laboratory) as the genus Salmonella have been sent to the Army Medical School, Washington, D. C., for complete type identification. In the 5 year period 1942-1946 Salmonella organisms from 370 cases have been studied (4). Of these, 151 came from outside sources such as stools from routine food handler examinations at army posts and material submitted by other hospitals. The information available on these cases is too limited for study purposes. The remaining 219 patients were observed in this hospital, the records of which furnished the data for the present investigation.

One hundred seventy-seven of the patients were males and 42 were females. Eighty-seven were in the 21-30 year age group. This predominance of young adult males is explained by the large number of troops here during the war years, and by the fact that sick Canal Zone employees are often hospitalized while other members of the family may remain at home with a transient illness. However, 50 of the cases were infants under 2 years of age, who were about evenly divided as to sex.

A total of 30 different types of Salmonella organisms were identified. In addition 3 more were recorded only as to the groups to which they belonged and a fourth only as a member of the genus Salmonella, the cultures dying before complete identification had been accomplished. For the purpose of this paper, the cases have been classified according to the predominating clinical manifestations as follows: Gastroenteritis, 160; Salmonella fever and septicemia, 13; and Salmonella carriers, 46. In table 1 the cases are classified on the basis of clinical and bacteriologic findings.

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TABLE 1

Two Hundred Nineteen Cases of Salmonella Infection Classified on the Basis of Predominating Clinical Manifestation and Etiologic Agent

ORGANISM	GASTROENTERITIS, 160 CASES	FEVER-SEPTICEMIA, 13 CASES	CARRIERS, 45 CASES
Group B			
<i>S. paratyphi B</i>	2		2
<i>S. typhi murium</i>	20	4	5
<i>S. typhi murium</i> var. <i>copenhagen</i>	2	2	2
<i>S. san diego</i>	5	1	1
<i>S. chester</i>	1		1
<i>S. reading</i>	1		
<i>S. saint paul</i>	4		
<i>S. zagreb</i>	1		
<i>S. abortus equi</i>	1		
<i>S. derby</i>	4		9*
<i>S. bredeney</i>	—		2
Unknown.....	2		
Group C			
<i>S. cholerae suis</i>	—	3	
<i>S. newport</i>	27	1	4
<i>S. newport</i> var. <i>puerto rico</i>	1		1
<i>S. montevideo</i>	33		3
<i>S. oranienburg</i>	5		3
<i>S. glostrup</i>	3		
<i>S. barcilly</i>	2		
<i>S. manhattan</i>	1		1
<i>S. oregon</i>	5		
<i>S. kottbus</i>	2		
<i>S. tennesse</i>	2		1
Group D			
<i>S. enteritidis</i>	3		3
<i>S. dublin</i>	1	1	
<i>S. panama</i>	9	1	2
<i>S. javiana</i>	3		
Unknown.....	1		
Group E			
<i>S. anatum</i>	15		3
<i>S. newington</i>	1		2*
<i>S. uganda</i>	3		
Group F			
<i>S. gaminara</i>	—		1
Unknown.....	—		1

* One carrier exhibited a double infection with *S. derby* and *S. newington*.

CLINICAL OBSERVATIONS

Gastroenteritis. In studying the characteristics of this group it was necessary to omit 18 cases who had other diseases which modified the clinical picture. Of the 142 accepted, 30 were infants under the age of 2 years, whose subjective symptoms could not be adequately elicited. The remaining 112 patients were mainly adults and will be referred to as the adult group. Their principal admission complaints were diarrhea, fever and abdominal cramps, followed closely, in order of frequency, by general malaise, nausea, vomiting, chills and headache. Table 2 shows the incidence of each of these complaints for this group, and of such objective complaints as the parents were able to observe in the infants.

TABLE 2

Admission Complaints of 142 Patients Suffering from Salmonella Gastroenteritis

COMPLAINT	112 ADULTS	30 INFANTS
Diarrhea.....	110 (98%)	30 (100%)
Fever.....	103 (92%)	27 (90%)
Abdominal cramps.....	97 (86%)	—
General malaise.....	71 (63%)	—
Nausea.....	70 (62%)	—
Vomiting.....	66 (58%)	14 (47%)
Chills.....	50 (44%)	—
Headache.....	48 (42%)	—

TABLE 3

Severity of Diarrhea in Salmonella Gastroenteritis, Showing the Greatest Number of Stools in Any Twenty-Four Hour Period

NO. OF STOOLS	112 ADULTS	30 INFANTS
Less than 5.....	29 (26%)	3 (10%)
5-10.....	51 (45%)	22 (73%)
More than 10.....	30 (27%)	5 (17%)

Diarrhea. The diarrhea usually started after a short period of fever and mild abdominal cramping, often accompanied by nausea and vomiting. It attained its greatest severity within twenty-four hours, during which time 72 per cent of the adults and 89 per cent of the infants passed 5 or more stools (table 3). These were most frequently described as watery and green. The diarrhea rapidly diminished after one to two days, so that body fluids and electrolytes seldom became dangerously depleted, although this occasionally did occur in infants. By the end of seven days the stools had returned to normal in 95, or 85 per cent, of the adults and in 20, or 67 per cent, of the infants. Of the remainder, the diarrhea was usually of a mild, intermittent type, continuing in some cases for five weeks.

Although gross blood or pus was rarely encountered, microscopic examination

of the stools frequently disclosed the presence of both. Blood was found in 20, or 18 per cent, of the adult cases and 12, or 40 per cent, of the infant cases, while pus was present in 27, or 24 per cent, of adults and 13, or 43 per cent, of infants. When detected, it was usually in the early stage of the disease, and disappeared as the stools resumed a normal appearance.

Fever. This symptom, often ushered in with a chill, was absent in only 9 adults and 3 children. The remainder demonstrated temperatures ranging from normal to 105°F., with a majority of the adults attaining a peak of 100.1 to

TABLE 4
Duration, in Days, of Fever in Salmonella Gastroenteritis

DAYS OF FEVER	112 ADULTS	30 INFANTS
None	9	3
1	11	4
2	16	4
3	24	5
4	21	7
5	13	1
6	11	2
7	2	—
8	2	1
9	1	2
10	2	1

TABLE 5
Maximum Leukocyte Count During Attack of Salmonella Gastroenteritis

WBC/CU. MM.	112 ADULTS	30 INFANTS
Under 8,001	41	4
8,001-10,000	35	5
10,001-12,000	11	7
12,001-14,000	8	5
14,001-16,000	7	3
16,001-18,000	4	3
Over 18,000	6	3

103°F. (oral) and a majority of the infants 101.1 to 104°F. (rectal). High temperatures were seldom sustained more than a few hours, but usually some fever persisted for a varying time up to ten days (table 4). Only 7 of the adults and 4 of the infants had fever longer than six days. The average duration of fever for the adults was 3.7 days, and for the infants 4.0 days.

White cell count. A leukocytosis often developed (table 5), more marked in infants than in adults, 36 per cent of the latter exhibiting counts under 8000. The differential count usually showed a shift to the neutrophilic side, but both the total count and differential rapidly returned to normal with the cessation of the diarrhea.

Agglutination. Fifteen of 20 serum agglutination tests were negative, the majority having been performed only against stock cultures of *S. paratyphi* B. However, 4 had been tested against the organism recovered from the patients' stools, on the eighth, tenth, thirteenth and sixteenth day of the illness, respectively. Of 5 positive tests, 4 were performed with the patient's own organism and 1 with the group representative. The earliest positive test was obtained twelve days after onset of the illness, with no history of a previous episode. The pertinent features of these cases are outlined in table 6.

Carrier state. Many cases yielded positive cultures only on the first day of hospitalization, and few were positive after the first week. However, at least 10 cases continued to excrete organisms after nineteen to thirty-five days of hospitalization, and were finally discharged as temporary carriers, after being warned against handling of food.

TABLE 6

Five Cases of Salmonella Gastroenteritis Exhibiting Positive Agglutination Reactions, Showing the Days After Onset of Illness that the Test was Performed, the Infecting Organism and the Antigen Used

CASE	AGE	SEVERITY OF ILLNESS	DAYS AFTER ONSET	INFECTING ORGANISM	ANTIGEN	TITER
L. L.	48	Severe	35	<i>S. paratyphi</i> B	Patient's own	1:2560
C. L.	37	Severe	5	<i>S. san diego</i>	<i>S. paratyphi</i> B	Neg.
			19	<i>S. san diego</i>	<i>S. paratyphi</i> B	1:320
T. D.	23	Mild	21	<i>S. anatum</i>	Patient's own	1:320
S. M.	59	Severe	12	<i>S. abortus equi</i>	Patient's own	1:640
D. C.	34	Mild	Weeks	<i>S. tennessee</i>	Patient's own	1:640

Epidemic cases. Included in the foregoing discussion were 27 cases of gastroenteritis due to *S. montevideo* occurring as an epidemic among soldiers in an Army camp (La Joya No. 2). Some of the cases became ill within six hours after ingesting the offending dish, while others noticed no symptoms for three days. Those falling ill early tended to have more fever and diarrhea than those affected later. One patient, suffering from headache, chills and fever, had no abdominal or bowel signs, but yielded positive stool cultures. The characteristics of the epidemic cases as a group, were similar in every respect to those of the sporadic cases.

Source of infection. Bread pudding served at the noon mess was found to be the cause of the epidemic cases at the Army camp. The source of its contamination was probably a temporary carrier working in the kitchen. Examination of the food handlers after the outbreak disclosed the organism in the stools of several, but these findings alone were inconclusive. The source of infection of sporadic cases could seldom be satisfactorily determined. Some had eaten a single meal in places whose sanitary conditions were questionable, while others were continually exposed to such conditions.

Monthly incidence. With the exception of the month of May, during which the 27 epidemic cases occurred, there was little monthly variation.

Treatment. This usually consisted in supportive measures to relieve the dehydration and to give symptomatic relief. Many patients received varying amounts of one or another of the sulfonamides, sulfadiazine and sulfaguanidine being the most frequently used, but the course of the disease was essentially the same as in those receiving no sulfa therapy.

Deaths. Three deaths occurred, all in infants. One, an infant of 4 months, had been ill for four weeks with diarrhea and vomiting. The stools were grossly bloody, and *S. saint paul* was cultured from them. Blood Wassermann was positive, and a syphilitic osteitis was noted. Despite therapy, which included sulfathiazole and parenteral fluids, the child became more dehydrated and died. Examination of the bowel at autopsy revealed only congested areas in the cecum and colon, and enlargement of the lymph follicles; peyers patches were not enlarged.

Another death occurred in a year old child brought in moribund with a temperature of 108°F. after severe watery diarrhea of two days' duration. Stools contained blood and pus, and *S. oregon* was isolated from culture. The child died a few hours after admission and no autopsy was permitted.

The third case, a 17 month old infant, died after a two weeks' illness consisting of diarrhea, fever and convulsions. The stools, at first watery, later contained blood and pus, and *S. panama* was isolated from culture. Spinal fluid was normal in every respect. The child received sulfadiazine, sulfaguanidine, and parenteral fluids, but became more dehydrated and febrile until death. Autopsy revealed superficial ulcerations of the esophagus, ileum and colon, cultures of which were negative for *Salmonella* organisms. It was the opinion of the pathologist that the ulcerations were not due directly to *Salmonella* infection.

Salmonella fever and septicemia. A total of 13 cases exhibited signs of extra-intestinal involvement. Blood cultures were positive in 7, spinal fluid in 3, pus from abscesses in 2, and peritoneal fluid in 1. *S. typhi murium*, recovered from 6 cases, was the most frequent offender, while *S. cholerae suis* was found in 3 and *S. dublin*, *S. panama*, *S. newport* and *S. sandiego* in 1 each. A few cases presented a typical typhoidal picture and others that of a septicemia, but some were difficult to class as one or the other. Three deaths occurred.

The characteristics of this group of *Salmonella* infections can best be demonstrated by a brief review of each case. Cases 1 and 2 have been reported separately elsewhere (5).

Case 1. Newborn male T. (590510), temporarily cyanotic after delivery, became jaundiced the next day. On the fifth day he passed a few green diarrheal stools. The same day respirations became labored and cyanosis reappeared. Blood counts and bleeding and clotting time were normal. X-ray revealed bronchopneumonia, and penicillin and oxygen were started. The cyanotic attacks became more frequent, and on the ninth day the baby exhibited moderate opisthotonus, the temperature rose rapidly to 105.6°F. (rectal), and the child died a few hours later.

Aseptic spinal puncture at the time of death yielded a yellow, viscid turbid fluid, culture

of which resulted in identification of *S. typhi* murium. Autopsy revealed a purulent meningitis, and a trilobulated heart.

Impression: Septicemia with meningeal localization.

Case 2. R. R. (582784) a 3½ month old girl was admitted because of muco-purulent nasal discharge and fever of two weeks' duration. There was no history of diarrhea. Signs of meningitis rapidly developed and spinal puncture yielded fluid of ground-glass appearance, containing 1,740 cells per cubic millimeter, 94 per cent of which were neutrophils. The organism cultured was *S. newport*. The next day diarrhea developed and persisted for five days. The same organism was cultured from the stools. The patient was treated with sulfadiazine and blood transfusions but the course was febrile and stormy for six weeks, during which time convulsions were frequent and 2 more positive spinal fluid cultures were obtained. The white blood cell count was only slightly elevated, the highest recorded being 11,550 per cubic millimeter, of which 64 per cent were neutrophils. During convalescence a left facial paralysis appeared, but cleared up rapidly, and the patient seemed well in every respect at the time of discharge, three months after admission.

Impression: Septicemia with meningeal localization.

Case 3. B. C. (540244) a 10 year old female came in with a history of chills and fever for five days and convulsions for twelve hours. Examination revealed slight neck rigidity. Spinal puncture yielded clear fluid which was found to contain only 2 cells and to present normal values for protein and sugar; culture was sterile. Repeated examinations of the blood for malaria were negative. The leucocyte count was 14,700 cells per cubic millimeter of which 75 per cent were neutrophils and 25 per cent were lymphocytes. The patient received sulfathiazole and parenteral fluids, but the temperature repeatedly spiked to 106°F. and the patient died two days after admission.

Autopsy examination revealed congestion of the pial vessels of the meninges, and slight mesenteric lymphadenopathy. Cultures from the blood and from the spinal fluid yielded an organism identified as *S. cholerae* suis.

Impression: Septicemia with beginning meningeal localization.

Case 4. E. B. (557619) a male, aged 3 years, came to the hospital complaining of pain in the right foot of two weeks' duration, and of pain in both hands and forearms of one day's duration. No previous illness was noted. Examination revealed local heat, swelling and tenderness of the painful parts. The white cell count was 11,000, with 60 per cent neutrophils and 40 per cent lymphocytes. X-rays at first showed soft tissue swelling but no bone involvement. Eight days after admission a fluctuant area on the dorsum of the left foot was aspirated, and culture revealed *S. typhi* murium. Autogenous agglutination was positive to a dilution of 1:640. Repeated blood and stool cultures were negative. Within a month after admission there appeared X-ray evidence of osteomyelitis of the entire left ulna, the entire right third metacarpal and the shaft and base of the left first metatarsal. During the first four months the patient exhibited periods of spiking temperature, and was treated with penicillin, sulfadiazine and blood transfusions. The lesions gradually healed by sclerosis, and the patient was discharged after five months of hospitalization.

Impression: Septicemia with multiple localizations in the bones.

Case 5. O. B. (562190) a 3 month old female was admitted because of swelling of the right knee of twenty-four hours' duration. There had been an episode of fever and diarrhea the previous week. Examination did not disclose the child acutely ill; the temperature was 100°F. and there was a fusiform, hot firm swelling above the right knee. Blood count showed 19,450 leucocytes per cubic millimeter of which 78 per cent were neutrophils and 22 per cent lymphocytes. The child was given sulfadiazine, and 2 days later incision of the swollen area revealed an osteomyelitis involving the lower end of the femur. Culture of the pus obtained at operation resulted in identification of the organism as *S. typhi* murium.

No blood cultures were made. Repeated stool cultures were negative until onset of a mild diarrhea one month after admission, at which time the same organism was cultured from the stool. During the first three weeks in the hospital the temperature rose intermittently as high as 102.4°F., then became normal and remained so. The leg gradually healed and the child was discharged on the fifty-third hospital day.

Impression: Septicemia with single localization in bone.

Case 6. D. L. (498363) a 5 year old female admitted because of chronic nephritis with nephrotic syndrome, had run a comparatively afebrile course until the fourth month of hospitalization, at which time the temperature rose to 104°F. and continued spiking daily. The white cell count gradually rose to 32,000 with a differential of 92 per cent neutrophils and 8 per cent lymphocytes. On the twenty-second day of fever a moderate diarrhea appeared and lasted five days. No stools were submitted for culture, but at the end of the diarrheal episode a blood culture was found positive for *S. typhi murium*. Two days later the abdomen, which had been continuously distended with ascitic fluid, began to protrude at the umbilicus, the center of the protrusion becoming thinned and pointed. A slight incision allowed the gushing escape of an estimated 1,500 cubic centimeters of milky fluid, culture of which was also positive for *S. typhi murium*. The abdomen became less tense but the chest filled with rales, a right pleural friction rub appeared, and the child died four days after the removal of the peritoneal fluid.

Autopsy revealed no free fluid in the abdominal cavity, but the peritoneum was covered with shaggy fibrinous exudate. The intestine was greatly distended, but no mucosal lesions were noted. Blood culture was positive for *S. typhi murium*, but cultures from the bowel, peritoneum and spleen were negative for *Salmonella*.

Impression: Septicemia with peritonitis.

Case 7. L. R. (543015) a 19 month old infant was admitted with a history of high fever and cough for eight days and vomiting for three days. The left lung field was filled with rales, and the white blood cell count was 13,600, with 73 per cent neutrophils and 27 per cent lymphocytes. A diagnosis of bronchopneumonia was made and administration of sulfadiazine begun. Because of a spiking temperature, repeatedly rising to 105°F. (rectal), a blood culture was made the day after admission, and yielded *S. typhi murium* variety copenhagen. A recheck blood culture and several stool and urine cultures were negative. Culture of sputum was not done. The temperature gradual fell to normal within a week, at which time the leukocytosis disappeared. No diarrhea was noted at any time. An X-ray showed the chest to be clear, and the child was discharged as well on the thirtieth hospital day.

Impression: Bacteremia with septic temperature and possible *Salmonella* pneumonia.

Case 8. R. V. (519870) a 1 year old female was admitted with a history of fever for two weeks and abdominal pain for four days. Slight diarrhea had been noticed a week prior to admission. Examination disclosed only a temperature of 101°F. (rectal), and a firm non-tender spleen palpable 2 centimeters below the left costal margin. The white cell count was 37,500 with 21 per cent neutrophils and 79 per cent lymphocytes. Chest X-ray was negative. Despite administration of sulfathiazole, the temperature remained at a level of 101-102°F. for the first nineteen days in the hospital. At the end of the first week the child began passing diarrheal stools, culture of which yielded *S. panama*. No blood cultures were reported, but stock cultures of *S. paratyphi B* were agglutinated by the patient's serum in a dilution of 1:640. On the nineteenth hospital day, the temperature dropped to normal, where it remained. The white cell count, which had been dropping slowly, became normal at the time the fever subsided.

Impression: *Salmonella* fever with intestinal involvement.

Case 9. H. B. (563025) a 56 year old male came in complaining of chills, fever and cough of three days' duration. Signs in the right lung field indicated pneumonia, and the spleen

was palpable. There was a leukocytosis of 11,500, with 93 per cent neutrophils and 7 per cent lymphocytes. Sulfadiazine was given, but the temperature remained at 100-101°F. At the end of a week the temperature suddenly rose to 105.4°F. and a mild transient diarrhea appeared. A blood culture taken at this time was found positive for *S. san diego*, but numerous stool cultures were negative. The white count, which had become normal before this episode, remained unchanged. After five days of spiking, the temperature dropped back to its original level where it continued for ten more days, then fell to normal. At that time one stool culture was found to contain *S. san diego*.

Impression: Salmonella fever with intestinal involvement.

Case 10. D. H. (543215) a 36 year old male complained of high fever and severe headache for fourteen days. There had been occasional chills, but no diarrhea or vomiting. Physical examination was negative except for a temperature of 105°F. The white cell count was 3,400, with 52 per cent neutrophils, 3 per cent eosinophils and 45 per cent lymphocytes. No malarial parasites were found on repeated examination of the blood, and the Widal test was negative. Two blood cultures were positive for *S. typhi murium* variety copenhagen, and the patient was treated with sulfadiazine. The height of the daily temperature fluctuations gradually diminished and after twelve days the temperature remained normal, and no more positive stool cultures were obtained. The patient's serum failed to agglutinate stock cultures of *S. paratyphi B*.

Impression: Salmonella fever with intestinal involvement.

Case 11. S. F. (574491) a 10 year old male came in with fever of eight days' duration. There had been no diarrhea, nausea or vomiting. The liver extended 3 centimeters below the right costal margin and the spleen 4 centimeters below the left. Both organs were tender to palpation. The white cell count was 6,400, with 50 per cent neutrophils and 50 per cent lymphocytes. No malarial parasites were found on blood smears. A chest X-ray was negative. The temperature was 104.2°F. on admission, and remained high for two days but gradually fell to normal by the end of a week. A blood culture taken the day after admission and another five days later were positive for *S. dublin*, but 2 subsequent cultures were sterile. Stool cultures, at first negative, became positive coincident with a mild diarrhea, which was present from the seventh to ninth hospital day. Over a four day period several positive stool cultures were obtained, after which all were negative. The splenic enlargement diminished, and the patient was discharged as well on the twenty-fifth hospital day.

Impression: Salmonella fever with intestinal involvement.

Case 12. E. B. (535954) a 59 year old male was admitted with fever and flank pain of two days' duration, which had appeared after a blow across the lower part of his back. Physical examination was negative, except for a temperature of 102°F. The white cell count was 7,700, with 77 per cent neutrophils and 23 per cent lymphocytes. Urinalysis was negative, and spinal fluid examination revealed no abnormality. X-rays of the abdomen, spine and chest were negative. A blood specimen taken the day after admission yielded *S. cholerae suis* on culture, and administration of sulfadiazine was begun. The temperature continued at 102°F., with an occasional brief rise to 105°F. A week after admission a moderate diarrhea appeared and *S. cholerae suis* was twice cultured from the stools. The temperature persisted at 102°F. and after a month, during which several sterile blood cultures were reported, another positive culture was obtained. Forty-seven days after onset of illness the fever subsided and further blood cultures were negative. During hospitalization the patient's auto-agglutination titer rose to 1:5120 and fell to 1:80.

Impression: Salmonella fever with intestinal involvement.

Case 13. A. M. (550711) a 19 year old male complained of headache, fever and left upper quadrant pain of five days' duration. He had had no diarrhea or vomiting, but there was a history of malaria one year previously. Palpation in the left upper quadrant caused pain,

but the spleen was not felt. The white cell count was 7,800 with 52 per cent neutrophils and 48 per cent lymphocytes. Blood examination for malarial parasites revealed *P. vivax*. Despite administration of atabrine the temperature rose gradually over forty-eight hours to 103.6°F. Further malaria smears were negative, but a blood culture taken on the fourth hospital day was positive for *S. cholerae suis*. During the next two days the temperature gradually returned to normal where it remained. Stool cultures were negative. Premature discharge prevented a satisfactory follow-up.

Impression: *Salmonella* bacteremia.

Carriers. The majority of these patients had entered the hospital because of some unrelated illness, and positive stool cultures were found during routine medical investigation. Of those with diarrhea, 6 were found to have amebic dysentery and 3 bacillary dysentery. Five others, all children with mild transient diarrhea, had respiratory and ear infections, which could well account for such a disturbance. A total of 8 healthy food handlers were sent to the hospital for further investigation after routine stool examinations had disclosed the presence of *Salmonella* organisms. None gave a history of illness suggestive of gastroenteritis. Stool cultures of all became negative within a week.

Twelve patients were able to recall episodes of gastroenteritis, only one of which had been proven bacteriologically to be due to *Salmonella*. Seven dated their trouble back only one month or less, while 5 had occurred five to seven months previously. Two of the latter had had no symptoms after the original attack, while 3 had continued to suffer from recurrent diarrhea.

No more than 2 positive stool cultures were obtained from each of 41 cases, 4 from 3 cases and 6 from 1 case, following which 3 or more negative culture were obtained before discharge. Only 1 patient continued to excrete *Salmonella* organisms after long observation. Admitted during an acute exacerbation of chronic cholecystitis, he had no bowel disturbance, but stool cultures consistently yielded *S. enteritidis*. Over a period of forty-three days 18 positive cultures were obtained, and he was finally discharged as a temporary carrier. No bile cultures were recorded.

Many of these cases received some type of sulfa therapy, others none. Between the two groups there was observed no significant difference in results.

DISCUSSION

Seligmann et al. (3) stated that in the United States 60 types of *Salmonella* organisms have been found pathogenic for man, the leading ones of which are *S. typhi murium*, *S. newport*, *S. oranienburg*, *S. montevideo*, *S. anatum* and *S. panama*. In the Isthmian cases 30 types were identified, the most common being *S. montevideo*, *S. newport*, *S. typhi murium*, *S. anatum*, *S. derby* and *S. panama*. Thus despite differences in climatic conditions, food and population makeup, between the 2 localities, essentially the same organisms were seen to prevail. Notably absent was *S. paratyphi* A. which previously had been observed as more common in warm zones (1). The seasonal incidence of *Salmonella* infections has been studied in Uruguay (6), Mexico (7), and the United States (3), and an increased frequency noted in the warm months. In Panama,

little monthly or seasonal fluctuation was seen, which is consistent with the even year-round temperature.¹

By far the most common manifestation of Salmonella infection was gastroenteritis. This diagnosis was made on the basis of typical clinical symptoms plus the demonstration of the organism in stool culture. Somewhat difficult to classify were those cases with only mild diarrhea or fever and positive stool culture. Each of these was carefully evaluated. As a rule, if another disease was present which could account for the symptoms, the case was considered a carrier. On the other hand, if no other disease was present and the symptoms subsided as the stool cultures became negative, it was classed as gastroenteritis. Cases of amebic dysentery with little fever, and bacillary dysentery, in which Salmonella organisms were found on stool culture, were considered carriers.

The onset of gastrointestinal symptoms was often preceded by a short period of headache, general malaise, chills and fever. One case during an epidemic experienced no other symptoms than these, but the diagnosis was proven by positive stool cultures. In the average case these symptoms, together with nausea and vomiting, were soon followed by mild abdominal cramping and abrupt onset of green watery diarrhea which seldom lasted longer than two days. During this time many adults were severely prostrated. The disease, however, tended to affect infants more severely, probably because their body fluids are easily depleted, and 3 deaths occurred in that group. Usually the fever and diarrhea subsided rapidly, the stools became formed, and the patient was well by the end of a week, the epidemic cases following the same course as the sporadic cases.

The fact is to be noted that moderate amounts of blood and pus were found by microscopic examination in the stools of an appreciable number of both adult and infant cases. While a few were grossly bloody, there was seldom a resemblance to the stools of amebic or bacillary dysentery, although Hormacche (6) in a study of infantile diarrhea noted types which he designated as choleric-form, dysentery-like and simple.

Agglutination reactions, even of the autogenous type, were variable and of little diagnostic help. Positive tests were obtained in some instances when the disease ran a protracted course, usually three weeks or more. Bornstein (1) has observed that where both stock- and auto-agglutinations may be negative, an "analytical Widal" utilizing "O antigen and separate H antigen for both phases . . . if a biphasic type is involved" may yield a positive test.

Extra-intestinal infections occurred about one-tenth as frequently as gastroenteritis. The prominent role of *S. cholerae suis* in such infections has often been mentioned, and was evident here, but *S. typhi murium* was the leading offender. *S. paratyphi* B, which together with *S. cholerae suis* accounted for

¹ Records of The Panama Canal, Section of Meteorology and Hydrography, show that there was a difference in temperature of only 2.8°F. between the warmest and coldest months, based on the bi-hourly mean over a thirty-four year period. The average temperature was 78.7°F.

over 50 per cent of the New York Salmonella Center's extra-intestinal cases (3), was not represented.

Although Bornstein (1) proposed dividing this group into septicemias, manifested by spiking temperature and tendency to localization, and fevers, manifested by a typhoid-like course, some cases did not fall readily into either class. Case 13 is an example, in which despite the patient's story of fever for five days before coming to the hospital, his temperature was normal on admission, then gradually rose to 103.6°F. and fell to normal over a period of four days. The organism recovered from his blood is known for its invasive quality, but no signs of localization were found. Sharp fluctuations were noted in the temperature curve of case 10. However, such fluctuation is seen in typhoid fever as the fastigium breaks, and since the patient had been ill for two weeks before admission, and exhibited low white cell count and absence of localizing signs, the case was regarded as a fever rather than septicemia. Some of the other cases of fever were not altogether typical, and the nature of the illness might easily have remained unknown had not a blood culture been made.

The cases of meningitis and osteomyelitis presented the normal features of infections of this type, and the route of infection was probably hematogenous, even though not proven by positive blood culture in each case. The difficulty of making an early diagnosis of meningitis in newborn infants is demonstrated by case 1. Also case 1, together with cases 5 and 8 illustrate the tendency for a simple gastroenteritis in infants to become a septicemia or fever. On the other hand, if the initial infection is systemic in character, it is not unusual for a mild diarrhea to appear during the course of the illness, as happened in several of these cases.

Salmonella organisms have been recovered from the sputum of patients exhibiting pneumonic involvement during the course of a systemic infection. In fact, it has been suggested that *Salmonella pneumoniae* be classed as a separate entity, but Bornstein (1) regarded it as a complication of Salmonella fever. Although case 7 may have been an example of this type, no sputum studies were made.

The findings in the carrier group were consistent with observations of other men, that the carrier stage is short lived, and that there are probably no healthy carriers. One possible exception is the person, mentioned by Bornstein (1), who lives in a house or institution where an outbreak of infection occurs. Although apparently not suffering from the disease, he may temporarily excrete Salmonella organisms in his stools. This may have been the explanation for positive stool cultures obtained from healthy mess personnel after the epidemic among the soldiers. In another instance a healthy lady was found to have stool cultures positive for *S. typhi murium* for a short time after her husband suffered an attack of gastroenteritis due to the same organism. Reliable agglutination tests in such cases, might help prove whether the person was an innocent bystander or the probable source of the original infection.

In the one instance where the organism was repeatedly cultured from the stool, a Salmonella cholecystitis may have existed. It is unfortunate that no bile studies were made.

Although Salmonella organisms are not usually excreted for long after an acute

attack, in a country where diarrhea is a common complaint, and cases are often not hospitalized, there must be many temporary carriers. Furthermore, as demonstrated in the epidemic, abortive attacks of Salmonella infection may occur without diarrhea, but with the organism excreted in the stools. These cases pass unrecognized in the absence of an epidemic, and serve as an additional source of infection. The existence of such sources, a warm climate and the unsanitary conditions that prevail in parts of the Isthmus combine to provide the necessary requirements to propagate the disease. Indeed, it is a surprising fact that epidemics are so infrequently seen.²

In cases of gastroenteritis, while mild symptomatic treatment is usually sufficient for adults, the fluid and acid-base balance must be closely watched in infants, and necessary measures taken to protect them. No valid conclusions can be drawn concerning chemotherapy. It should not be used until the patient is well hydrated, but in infants, especially, it may offer some protection against development of a septicemia. In cases of meningitis it has been demonstrated that sulfonamides may aid recovery (5). Sulfadiazine is probably the drug of choice. It is doubtful if it is of any use in the carrier state.

Streptomycin was not used in any of these cases. The few reports of its use in this type of infection are inconclusive. If it does prove of value, it will place an added premium on early recognition and treatment of Salmonella infections.

SUMMARY

A study has been made of 219 cases of Salmonella infection observed in Gorgas Hospital, on the Isthmus of Panama, in the five year period 1942-1946. The various manifestations of the infection have been presented and discussed.

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² Health Department Records show only two other epidemics of Salmonella infection on the Isthmus. The first occurred at Ft. Amador in January 1931, with 40 soldiers suffering from typical gastroenteritis. The organism isolated from stool cultures was studied by E. O. Jordan, who recognized it as a new type and designated it as *S. panama*. His complete report of the episode appeared in *J. Infect. Dis.*, 55: 224-227 (Sept.-Oct.) 1934.

The second outbreak, in July and August 1940, involved 16 persons at Ft. Clayton. The organism recovered from the stools was identified as *S. schottmülleri* variety java by the National Salmonella Center at the University of Kentucky.

CARRION'S DISEASE

A STUDY OF THE INCUBATION PERIOD IN THIRTEEN CASES

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INTRODUCTION

The duration of the period of incubation of Carrion's disease has been a most controversial subject. Prior to the unfortunate death of Carrion in 1885, it was not known. The symptoms were always referred to the onset of the eruption of the verrugae, or, what is called today, the preeruptive stage of the disease. Different authors have reported evidence indicating the duration of this period to be variable. Tupper (1) in 1877 thought that the period of incubation fluctuated from a few days to several months. Carrion (2) considered it as 8 to 40 days in duration. Antunez (3) reported 7 to 21 days, Aree (4) from 12 to 40 days and Odriozola (5) 15 to 40 days. Odriozola denied the possibility that the incubation could be of many months duration, an opinion shared by other authors (6). It should be noted that cultures for *Bartonella bacilliformis* were not obtained in these previous studies.

The present study is an attempt to give further information on the period of incubation of Carrion's disease. This study was carried out at different hospitals in Lima during the period 1938-1942.

All of the cases at the initial onset of symptoms proved to be due to *Bartonella* by the demonstration of the organism in blood cultures, using the Geiman medium (7) or in smears of the peripheral blood when anemia had developed.

PRESENT STUDY

The period of incubation was studied in a series of 13 cases of Carrion's disease. All the patients were perfectly healthy before becoming exposed in the endemic areas to the bite of the *Phlebotomus*, transmitter of the disease. These cases have been divided into two groups. In the first there were 7 cases which remained in endemic zones for only a few hours to three days. In these the period of incubation was quite accurate; an almost experimental observation.

The extremes in incubation period varied from 20 to 23 days in 4 cases, while in one case it was as long as 100 days.

Two typical examples follow:

E. B., a single, 31 year old, Peruvian cattle-trader, in July 1939, made a trip on foot from Lima to Huanayo, a city in the Andes. On the 6th and 7th of July, he passed through an area where Carrion's disease is known to be endemic. On the 28th of July, the patient developed symptoms, which he called "flu". He had slight fever, malaise, a slight yellow discoloration of the skin and sclera, dark urine, pains in the bones, polydypsia and anorexia.

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The patient was also found to have anemia with *Bartonella bacilliformis* in the red cells of peripheral blood. This vague symptomatology persisted for approximately one month and then subsided. On October 3rd, he noticed an eruption of verrugae in the skin.

J. R., a single, 22 year old, white Peruvian, without any significant past medical history, on the night of February 20th, while traveling from Huancayo to Lima in a truck, stopped for a few hours in an area where Carrion's disease is endemic. He remained in Lima in very good health until the first of April, 1941. Suddenly on the afternoon of that day he developed a chill followed by high fever. He felt tired and exhausted and was unable to do anything that required effort. He complained of "sleepiness of the lower extremities", epigastric distress, polydipsia and anorexia. During the following ten days, the patient

TABLE I

CASE NUMBER	DURATION
	<i>days</i>
1	21-22
2	40
3	20-23
4	100
5	20
6	20
7	88-90

TABLE II

Second Group: Probable Incubation Period

CASE NUMBER	DAYS OF RESIDENCY IN ZONE WHERE THE DISEASE IS ENDEMIC BEFORE SYMPTOMS
8	28
9	21
10	30
11	30
12	30
13	19

developed an anemia, urine of dark wine color and delirium. After hospitalization, he was found to have a red blood cell count of 3,000,000 and white blood cell count of 11,000. Very many of the red blood cells contained *Bartonellae*. He died 10 days after admission with a complication of bacteriologically proven *B. typhosus* infection.

In the second group of 6 cases (Table II) the period of incubation could be obtained only with approximate accuracy. The day of arrival in the endemic zone was known but since the patients remained there, it was not possible to determine exactly when the infection occurred. In these cases the incubation possible varied from 19 days to 30 days which indicated that infection occurred during first exposure in the endemic area.

DISCUSSION

The difficulty encountered previously in establishing an accurate incubation period for Carrion's disease is due primarily to the very limited number of

observations, the lack of reliability of the patient's histories, and the fact that the disease frequently has a varied symptomatology. Occasionally, the disease is not associated with subjective symptoms during the whole course, manifesting itself only by an eruption of verrugae in the skin. MacKehenie (8) showed the marked variability that can occur in different patients. He remarked that there were subclinical cases in which, without any symptoms suggesting the disease, it is possible to obtain blood cultures positive for bartonella.

The atypical syndrome during the invasive stage includes such symptoms as headache, pain in the bones and joints, moderate fever lasting for a few days without any anemia, etc. In many cases such vague, indefinite symptoms are interpreted by the patients as "flu", "gastrointestinal upsets", and are not recorded as the initial stage of the disease. Blood cultures allowed us to detect the *Bartonella bacilliformis* in these apparently non-specific episodes. In bartonella anemia (Oroya Fever), continuous fever, marked anemia and slight jaundice, with *Bartonellae* in the peripheral red blood cells permits an early diagnosis.

It becomes evident from the present study that the disease has a variable incubation period. In the first group of seven cases who remained in endemic areas only from a few hours to three days, the incubation period varied from 20 to 100 days. The period of incubation fluctuated from 19 to 30 days in the second group of six cases in which it was possible to know only the days of residence in zones where the disease is endemic before the onset of symptoms. These findings are in agreement with experimental human inoculations. The first made by Carrion himself indicated an incubation period of 21 days (9). The second was a self-inoculation made by Kuczinski-Godard, who observed initial symptoms 17 days later.

SUMMARY

The period of incubation of Carrion's disease studied in a group of 13 patients varied from 19 to 100 days.

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DIVISION OF TROPICAL DISEASES, NATIONAL INSTITUTE OF HEALTH

To conduct an intensified tropical disease research program, the United States Public Health Service has established a new Division of Tropical Diseases in the National Institute of Health, Dr. Thomas Parran, Surgeon General, announced on May 5, 1947.

The new division will expand the research studies formerly pursued by the Zoology Laboratory which it supplants. It will be responsible for work in the fields of tropical medicine, medical zoology, and parasitology. Malaria research, now going on in the Divisions of Infectious Diseases and Physiology, is transferred to the Division of Tropical Diseases.

Heading the new unit is Scientist Director Willard H. Wright, former Chief of the Zoology Laboratory and nationally known expert in tropical disease research. He was recently awarded the Legion of Merit for his work as Field Director, Commission of Schistosomiasis, U. S. Army, in the Southwest Pacific and Japan. A native of Findlay, Ohio, Dr. Wright received a D.V.M. degree from George Washington University in 1917, an M.S. from American University in 1931, and a Ph.D. from George Washington University in 1935.

Dr. Wright is affiliated with the American Society of Tropical Medicine, the American Academy of Tropical Medicine, the American Society of Parasitologists, the American Public Health Association, the Helminthological Society of Washington, Washington Academy of Science, and the New York Academy of Sciences.

"MAL DEL PINTO" OR "CARATE" AND ITS TREATMENT WITH CHLORHYDRATE OF 3-AMINO-4 OXIARSEN BENZEN (MAPHARSEN)¹

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The purpose of this article is to give a brief presentation of our observations made on "Mal del Pinto" and its treatment with chlorhydrate of oxophenarsen (Mapharsen) at the Hospital of Arcelia, Guerrero (indicated in map).

According to the first census made of "Mal del Pinto" (Dept. of Public Health, 1934) there are 270,049 (without the "pintides") persons affected with the disease in the Republic of Mexico. We have used this figure as a base for our calculation of index numbers listed below and in the map (Fig. 1).

STATES	PER CENT	INDEX NUMBER
México.....	34.67	100.0
Guerrero.....	23.67	68.3
Michoacán.....	23.15	66.8
Oaxaca.....	16.98	49.0
Puebla.....	11.19	32.3
Chiapas.....	7.55	21.8
Morelos.....	2.29	6.6
Tabasco.....	1.94	5.6
Nayarit.....	0.90	2.6
Campeche.....	0.39	1.1
Jalisco.....	0.36	1.0
Quintana.....	0.18	0.5
Veracruz.....	0.13	0.4
Colima.....	0.06	0.2

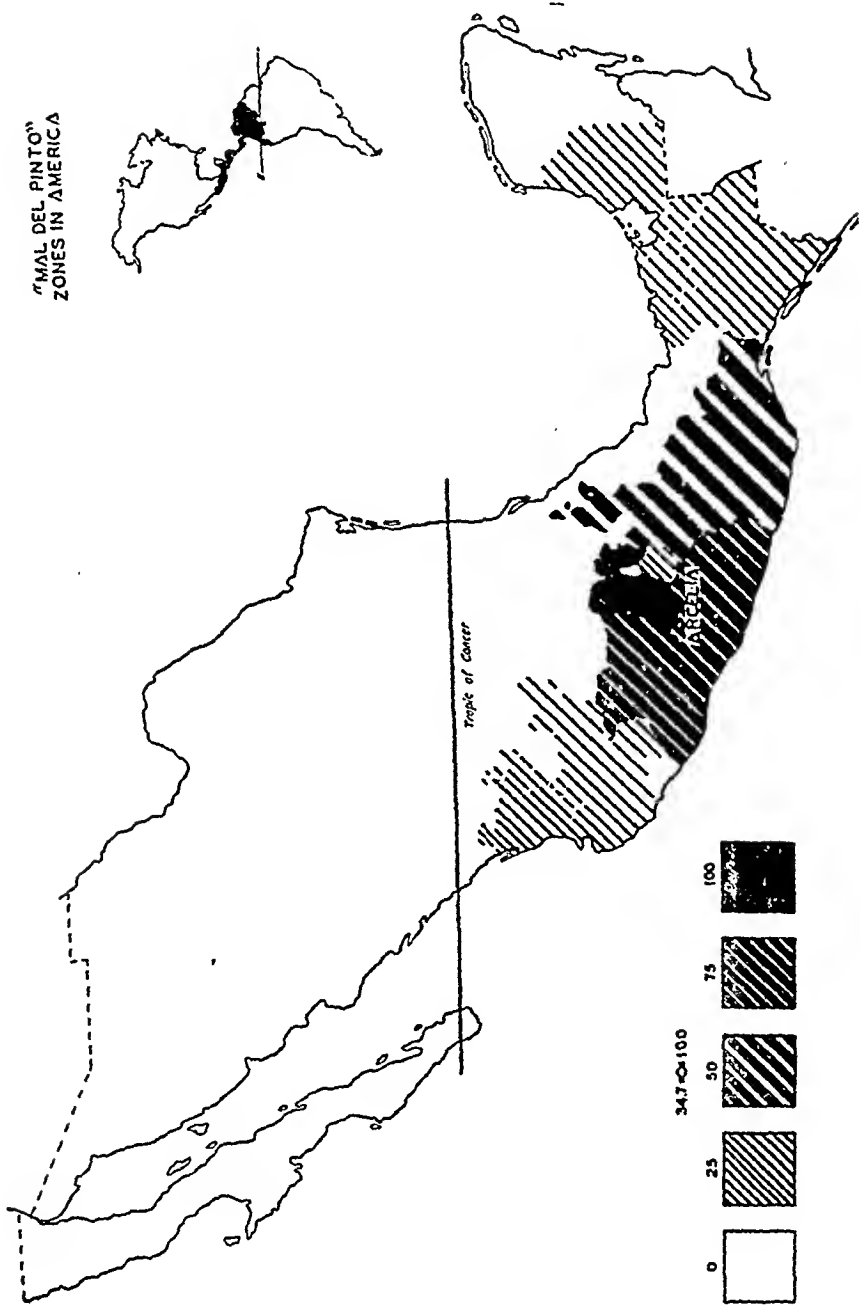
There are approximately 500,000 people infected with Pinto in the rest of the Western Hemisphere especially in Colombia (Peña Chavarría and Shipley, 1925), Venezuela (Iriarte, 1940), Ecuador (León Luis, 1940) and Central America. It seems probable that this disease was prevalent among the ancient people of this Hemisphere.

Etiology. Saens, Grau and Alfonso (1938) published the discovery of a treponema in the lesions and lymph glands of Pinto patients. According to Leon Blanco (1938) this discovery was made by Alfonso, Grau and himself.

Brumpt (1939) called this organism *Treponema carateum* and later Leon Blanco (1939) suggested the name of *Treponema herrejoni*.

Varela and Nieto (1940) found that the *Treponema carateum* is immediately paralyzed by the action of bile, while *Treponema pallidum* becomes immobile only after about thirty minutes (Noguchi, 1928).

¹This work was performed with the economic support from the Dirección of Inter-American Affairs and that of the C.I.C.I.C.



REPUBLIC OF MEXICO. DISTRIBUTION OF 'MAL DEL PINTO'.

We have not been able to grow *Treponema carateum* in anaerobic media, or on the chorioallantoid membrane, or in the yolk of fertilized eggs.

Working with Dr. Pomerat (of the Medical School of the University of Texas, Galveston) and using his simplified technic of tissue cultures, we obtained an abundant growth of treponema in cultures of nervous tissues of mouse embryos. The inoculation of the cultures into the normal skin of a Pinto patient gave an allergic reaction in ten minutes, and the initial Pinto lesions did not appear in the man during a six month period of observation.

Mooser, Varela and Vargas (1936) and Briceño Rossi and Iriarte (1944) were not successful in their attempts to reproduce Pinto in laboratory animals. Curbelo and his collaborators (1938) produced keratitis and orchitis in rabbits inoculated with the treponema of a case of Cuban Pinto; but according to Leon Blanco (1945) this was an error and the lesions were produced either by common microorganisms or *Treponema pallidum*. Leon Blanco (1945) describes the positive intradermal inoculation of a rabbit and the transmission of the infection to man and the development of the initial lesions of Pinto. We have not been able to reproduce this experiment after various attempts in which we have used Pinto patients showing treponemata in abundance.

The experimental transmission of Pinto to man was obtained by Tellez (1899). Leon Blanco (1942) demonstrated that Pinto patients are neither immune to reinfection during the disease nor after recovery. This same author mentions the lack of cross immunity to syphilis in accidental infections.

Padieha (1946) made nine inoculations of yaws patients with "Pinto" and thinks that there is partial cross immunity.

The first experiment on the transmission of Pinto by Simuliidae was made by Juan J. León (1860). González Herrejon and Ortiz (1938) found treponema in the digestive tract of *Simulium haematopolum* which had fed on lesions of Pinto patients, but Leon Blanco (1940) did not succeed in transmitting the disease with these insects.

Leon Blanco and Soberon (1941) pointed out the possibility of transmitting Pinto by *Hippelates*, Leon Blanco (1940) found *Treponema carateum* in the secretion of crevasses in fissures and in the sweat of patients infected with Pinto. These findings gave support to the opinion of its possible transmission by direct contact.

In the zone around Arcelia, Guerrero, we studied 805 individuals with relation to past history of Pinto in their parents. The data obtained are as follows:

Descendants from "Pinto" patients

	STUDIED	PINTO PATIENTS	PERCENTAGE
Offspring: one or both parents affected with Pinto . .	575	323	56.2
Offspring: parents not affected with Pinto.....	230	100	43.5
Total.....	805	423	52.5
Difference in percentages.....	12.7	2.6	0.01

Characteristics of the Disease. "Mal del Pinto" generally begins with spots of desquamating erythema as has been mentioned by Corona (1811), these lesions were called "pintides" by Latapí and Leon Blance (1940). Among two hundred patients studied at the Hospital of Arcelia, these "pintides" appeared

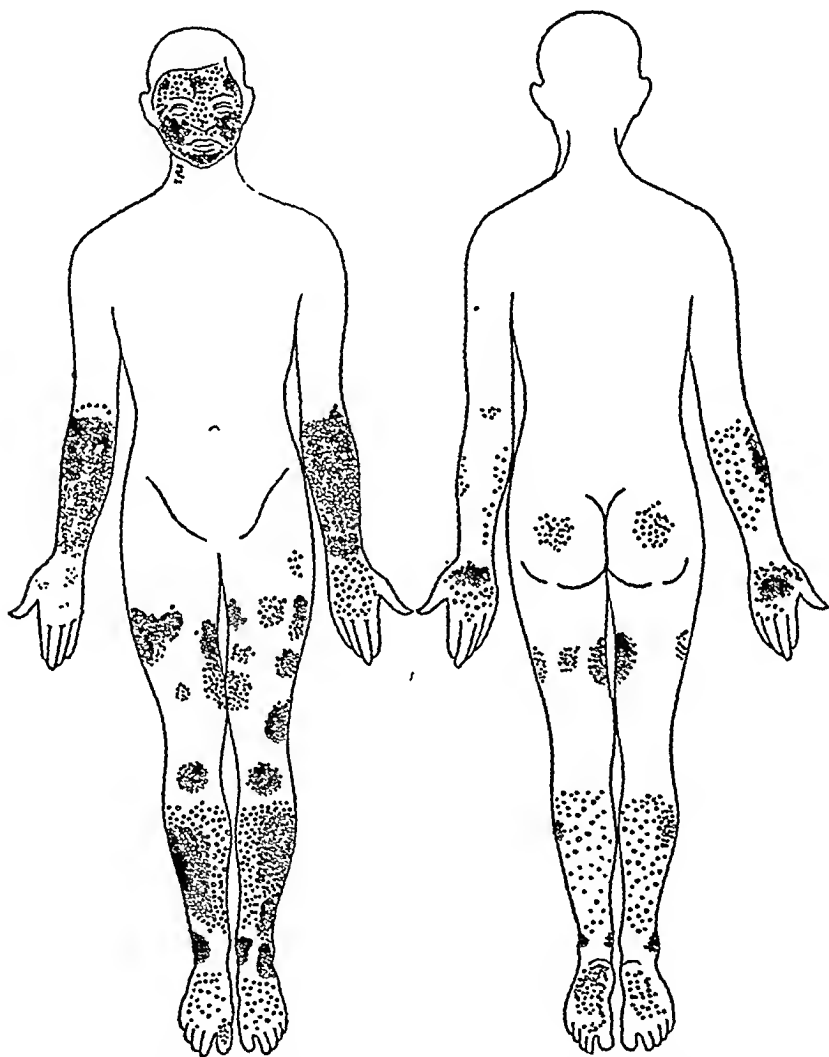


FIG. 1. LOCALIZATION OF THE INITIAL ERYTHEMOSCAMOUS LESIONS (PINTIDES) IN 200 PATIENTS WITH "MAL DEL PINTO". ARCELIA HOSPITAL, GUERRERO, MEX.

particularly on the uncovered areas of the body i.e. the face, arms and legs (fig. 1).

Later the dyschromic lesions appear, which were classified by Berecochea (1811) according to their color in red, pink, blue, brown and white.

From observations made on two hundred patients (fig. 2) we can say that the red lesions of this disease are distributed practically all over the body, but they are most frequent on the face, the extremities and the shoulders. The blue

spots are located in the same areas as the red ones (fig. 3) and the white spots are mainly found on shoulders, elbows, hands, knees and feet, as well as scattered over the rest of the body (fig. 4).

In ninety six (48.5%) out of two hundred patients observed during the dyschromic stage pruritus was present, and three of them had aches in their bones

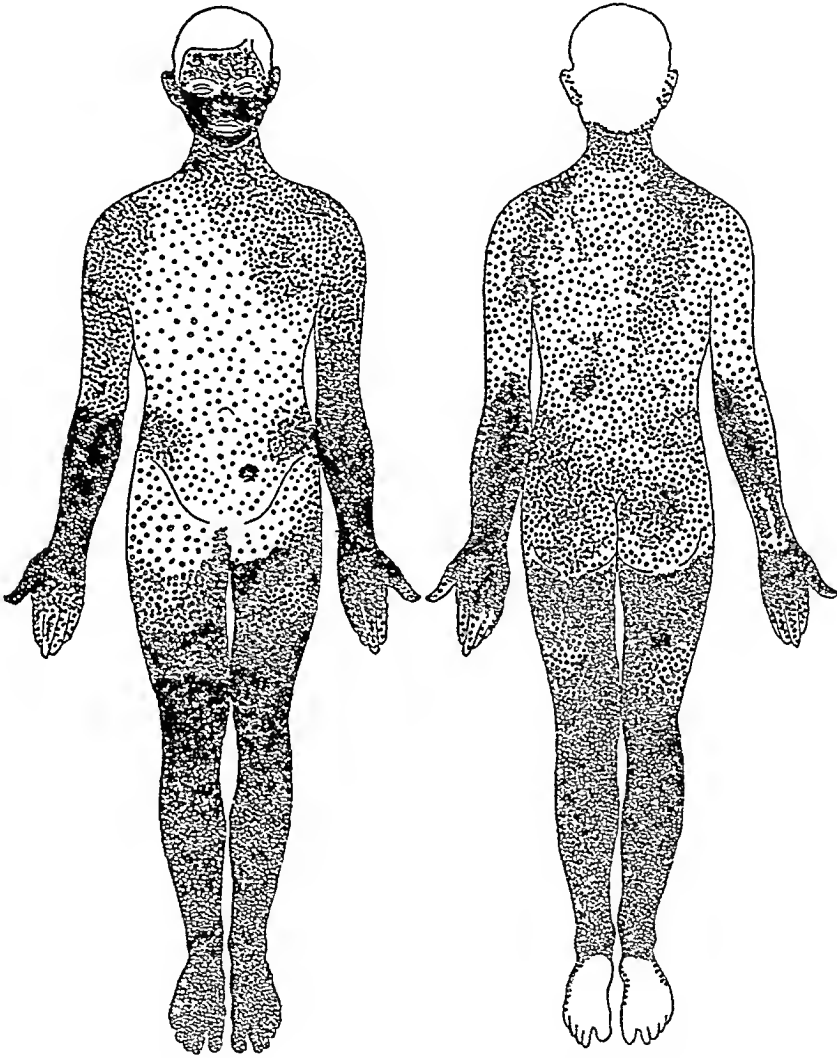


FIG. II. LOCALIZATION OF THE RED ZONES IN 200 PATIENTS WITH "MAL DEL PINTO".
ARCELIA HOSPITAL, GUERRERO, MEX.

which at times were very severe. Eighty-four (42%) out of two hundred patients had frequent palpitations, and the maximum and minimum blood pressure had a marked tendency towards figures lower than those normally found in México.

Aortic lesions in patients affected with "Mal del Pinto" have been described

by Thonard and Brewster in Colombia (1940), Aguirre Pequeño (1944) and Varela (1945) in México.

Menk (1926) obtained a high percentage of positive Wasserman reactions in Pinto patients. Escobar (1940) and González Gusmán (1940) found that Kahn verification tests were also positive in those patients. Varela, Olarte and Castro

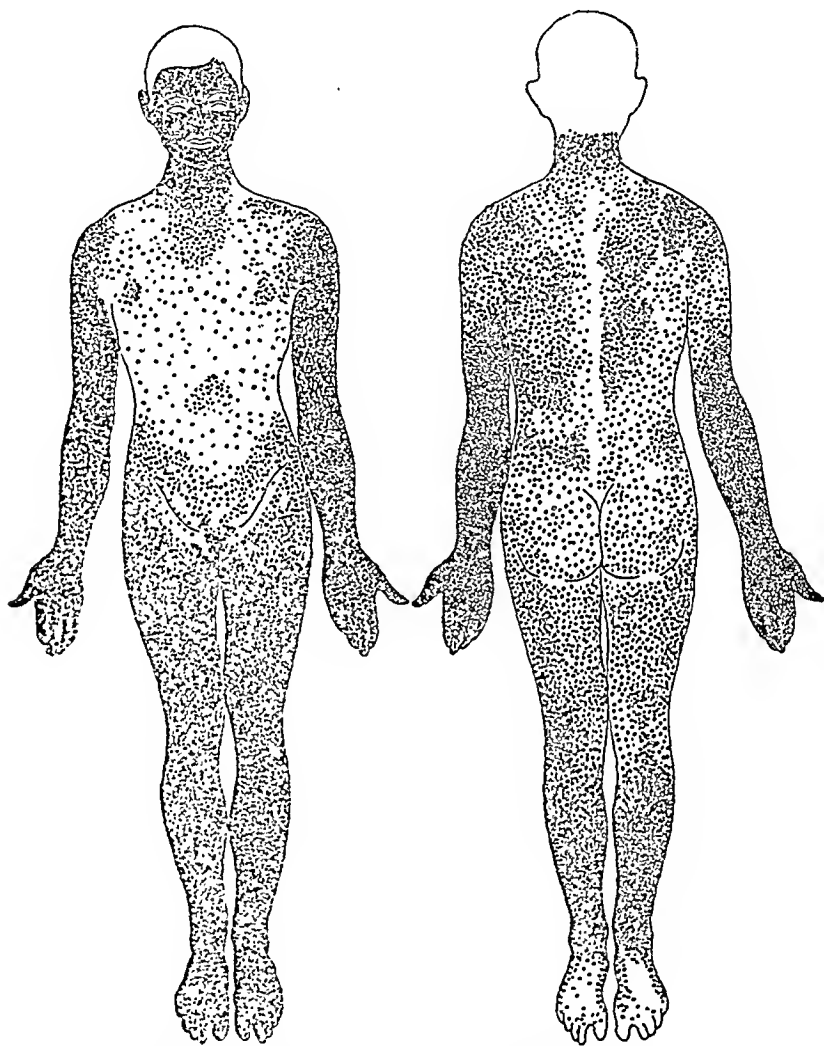


FIG. III. LOCALIZATION OF THE BLUE STAINS IN 200 PATIENTS WITH "MAL DEL PINTO". ARCELIA HOSPITAL, GUERRERO, MEX.

(1945) found that in the Kahn triple quantitative reaction (1942 and 1943) the positivity increases with the greatest concentrations of sodium chloride and that the globulins and pseudoglobulins of the blood serum of Pinto patients react in a similar way, both giving positive reactions with Kahn Standard tests.

Uribe Escobar (1929) emphasized the difficulty in modifying the positivity of the serological luetic reactions in Pinto patients after treatment. Other

investigators have since confirmed this fact, and in our studies in Arcelia only three out of sixty-six patients showed an evidence of decreasing the intensity of the Kahn reaction.

Those who have recently made a study of the cerebrospinal fluid (Pelaez

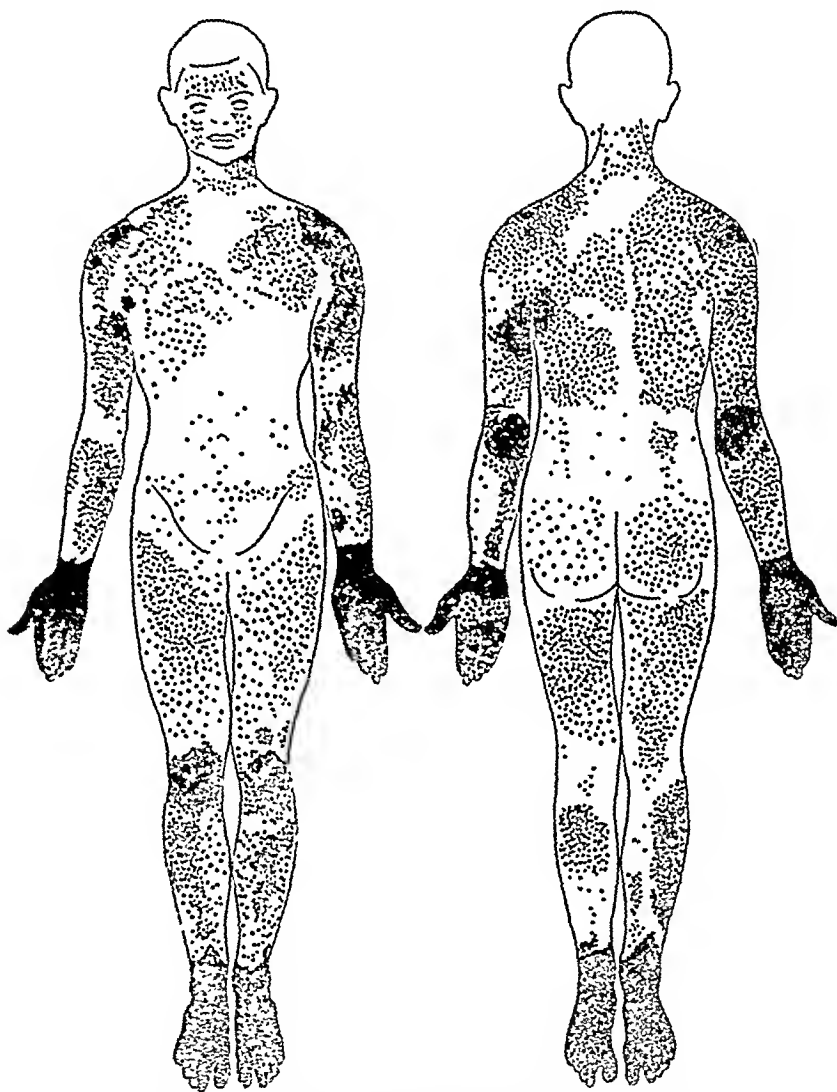


FIG. IV. LOCALIZATION OF THE ACROMIAS IN 200 PATIENTS WITH "MAL DEL PINTO".
ARCELIA HOSPITAL, GUERRERO, MEX.

Botero 1939, Leon Blanco 1942, Varela 1945) found no alteration whatsoever in the fluid of Pinto patients.

Treatment by Arsenicals. Gratz (1913) in Colombia first employed arsenobenzol in the treatment of "Mal del Pinto o Carate". Leon Blanco (1942), used with good results, chlorhydrate of the oxide of meta-amino-para-hydroxyphenylarsine (Mapharsen).

The doses of "Mapharsen" administered by Leon Blanco (1942) were as

follows; the first one of 0.02 grams for women and 0.03 grams for men, repeated after a lapse of four days; if no symptoms of intolerance appeared, he gave 0.06 grams to men and 0.04 grams to women after a period of one week.

In the Arcelia Hospital we used the same dosage for men and women injecting 0.04 grams of "Mapharsen" twice a week. The minimum number of injections administered was five and the maximum fifteen, the latter number having been used in the majority of cases.

Out of several hundred patients who were treated, sixty-six were observed for periods up to one year.

It was found that in six of them the lesions were not changed in aspect and in the remaining, only part of the hyperchromic spots disappeared.

SUMMARY

Summarizing our observations we can say that about 700,000 people in this hemisphere are affected with "Mal del Pinto o Carate".

The study of 805 people in the pinto area around Arcelia, Guerrero shows that 56.2% of the children of parents affected with Pinto have the same disease, and that healthy parents have Pinto children in a proportion of 43.5%. (Difference in percentages = 12.7 - 2.6).

We report the possibility of obtaining growth of *Treponema carateum* in cultures of embryonic nervous tissue.

These cultures caused allergic reactions in a Pinto patient but did not produce infections in men.

The distribution of Pinto lesions is shown in groups of 200 patients (figs. 1, 2, 3, 4). The maximum and minimum blood pressures of these cases have a tendency toward low numbers.

The treatment of Pinto with "Mapharsen", administered in 15 injections of 0.04 twice a week gave some results in 96% of the cases. The intensity of the Kahn reaction diminished in the blood serum of 4% of the cases.

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SOME FACTORS THAT INFLUENCE THE DEGREE OF PARASITEMIA IN DUCKS INFECTED WITH *P. LOPHURAE*¹

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The problem of the mechanism of immunity in malaria has been studied extensively both in man and in experimental animals, however, today it is not satisfactorily explained. No doubt this problem is complicated by the variations in reaction produced in man by the different species of plasmodia and furthermore by the difficulty of transferring information obtained from animal experimentation to man. Since there is a wide variation in the response of different host to different plasmodia we will consider only the problem of the mechanism by which a rapid diminution occurs in the number of parasites following the peak of parasitemia in ducks infected with *P. lophurae*.

The course of an infection produced by *P. lophurae* in ducks has been established by the studies of different investigators (1-3). It has been shown by Hewitt and associates (1, 2) that the course of the disease is influenced by the age of the bird and the number of parasites used for the inoculum. In our studies the ducks, 2-4 weeks of age, when given a lethal inoculum, die at one of two periods in relation to the peak of the parasitemia. A majority of the birds die when the number of parasites are at their peak. The second largest number succumb during the period in which the parasites are decreasing. Only a small percentage of these ducks with a high degree of parasitemia survive the infection. In this latter group the degree of parasitemia may reach 400 parasitized cells per 500 red blood cells by the 5th day of the infection. The number of parasites then rapidly decrease until 48 hours later at which time only 5-10 parasitized cells may be found within 500 red blood cells. These ducks rapidly return to normal and usually within an interval of 5-7 days show no abnormalities in their hemogram (4, 5).

Phagocytosis certainly plays a role in enabling a host to rid itself of plasmodia. Ben Harel (6) considers that phagocytosis plays the significant role in immunity in malaria, and says, "It would therefore seem probable that the great reduction in the number of parasites is due to their actual destruction by fixed tissue cells, as well as by circulating phagocytes . . . Phagocytosis is probably the most important factor in causing the disappearance of the parasites from the peripheral circulation." Hewitt (7) in his monograph on Bird Malaria published in 1940 reviewed the previous studies on immune reactions and concluded that "a pre-

¹ This work has been aided by a grant from the John and Mary R. Markle Foundation. Research paper No. 843 Journal Series, University of Arkansas. Presented at the Duke Medical Alumni Reunion at Durham, N. C., April 24-26, 1947, by R. H. Rigdon.

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cipitous drop to the number of parasites immediately following the crisis is not a constant feature of bird malaria infections . . . It is therefore by no means conclusively established that acquired immunity is developed suddenly but it seems more likely that phagocytosis, in combination with other immune mechanisms increases in degree throughout the acute rise of infections and continues to rid the host of parasites after the parasite peak has been reached." Cannon and Taliaferro (8) in 1931 reviewed the literature and investigated the problem of immunity in canaries infected with *P. cathemerium*. They observed a rapid decrease in the number of parasites following the crisis. This problem was discussed by them from the standpoint of the sudden mobilization of antibodies and the immediate increase in the number of phagocytes. Recently Gajewski and Tatum (9) stated that, "There is very little direct evidence that a protective antibody is present in the blood during acquired immunity to *P. cathemerium* and *P. relictum* which functions in keeping the blood practically free of parasites during latency. It is certain, however, that the parasite or one of its metabolic products sensitizes or increases the functional capacity of the phagocytic cell." Pathological studies (8) on ducks infected with *P. lophurae* show that the cells of the reticulo-endothelial system progressively become filled with malarial pigment before the peak of parasitemia is reached. Anatomically it is difficult to see how these already filled endothelial cells could remove from the circulating blood the tremendous number of parasitized cells present at the time of the peak of the infection.

In considering this problem of the mechanism by which the number of parasites decrease following the peak of infection it is significant to know the factors that increase the number of parasites in the blood of the duck. Rigdon and Rostorfer (11) have shown that the number of parasites are markedly increased in ducks with a polycythemia. If the immune mechanism is not diminished in ducks kept within a decompression chamber it is difficult to appreciate the fact that the total number of parasites parallel the increase in number of red blood cells.

A second factor which has been found to increase the number of parasites in ducks is the transfusion of red blood cells (3). The decrease in the number of parasites which follows the peak of the infection can be inhibited by the frequent intravenous injection of duck red blood cells. The length of life of infected ducks can be prolonged by these transfusions. It is difficult to understand the immune mechanism by which the number of parasites decrease in a duck while the parasitemia increases when red blood cells are added to the circulation. It has been suggested that the number of parasites may be kept at a high level provided a sufficient quantity of blood is injected to keep the total erythrocyte count within the range of normal.

A third factor which has been found to increase the number of parasites in ducks is oxygen (12, 13). Ducks infected with *P. lophurae* and placed in an oxygen chamber with a concentration of 30-80 per cent oxygen show a greater number of parasites in the peripheral blood than control birds. As far as we know there are no studies to show that an immune mechanism is inhibited when the host is kept in the presence of an increased concentration of oxygen.

It is of interest to observe that *P. lophurac* are not as likely to enter young red blood cells as they are to enter adult erythrocytes. There is a marked diminution in the number of adult red blood cells when the parasitemia is high. This is accompanied by an increased number of young erythrocytes. Why the preference of these plasmodia for the adult red blood cell is not known. It has been shown that the young red cells have only a small amount of hemoglobin and furthermore the oxygen-carrying ability of the blood decreases during the course of the infection (14). These observations would suggest that probably either the deficiency of hemoglobin in the red cells or the decrease of oxygen carried by the cells makes an unsatisfactory environment for the propagation of these plasmodia.

Recently there has been observed certain *in vitro* conditions that were detrimental to *P. lophurac*. Since some of these may occur in the infected duck it may be significant to review them at this time (15). Malarial infected blood when treated by bubbling carbon dioxide through it, fails to produce the same degree of parasitemia when injected into ducks as infected blood similarly treated with either oxygen or nitrogen. The greater injury to the plasmodia occurs when the carbon dioxide is bubbled rapidly through the blood. The mechanism of this injurious effect of carbon dioxide upon these plasmodia is not known.

With regard to the *in vitro* observation of the effect of carbon dioxide on *P. lophurac* it should be recalled that plasmodia consume a large amount of both glucose and oxygen when grown *in vitro* (16, 17). Wendel (17) has suggested that, "The parasite very probably depends upon the host for disposal of end-products of its metabolism. Such products, if allowed to accumulate, might very quickly destroy the parasite. Indeed, we have noted that parasites in defibrinated blood to which nothing has been added undergo such extensive morphological changes in the course of 6 to 8 hours of incubation as to suggest that most of the parasites are no longer alive."

Since there is evidence from the *in vitro* experiments to indicate that carbon dioxide is detrimental to *P. lophurac* a group of infected ducks were studied to determine if there were any variations in the amount of carbon dioxide within the plasma during the disease. The carbon dioxide combining power was determined on the same samples of plasma. Blood for these studies was obtained from the heart at different periods of the infection and kept under paraffin oil. Heparin was used as the anti-coagulant. The gasometric method used for the determination of the carbon dioxide was that outlined by Peters and van Slyke (18) for the volumetric blood gas apparatus.

The average carbon dioxide combining power of the plasma on 12 normal ducks is 51.4 volumes per cent. The average carbon dioxide content of the plasma on 21 normal ducks is 46.9 volumes per cent. The observations on the carbon dioxide combining power of the plasma of 61 ducks with malaria and the carbon dioxide content of the plasma of 44 ducks with malaria are shown in figure 1. From these data it is obvious that *P. lophurac* infected birds develop a severe acidosis. The degree of this acidosis apparently is directly related to the degree of anemia. The carbon dioxide content of the plasma, however, remains ap-

proximately normal until the anemia falls below one million cells at which time the carbon dioxide content of the plasma progressively decreases until death.

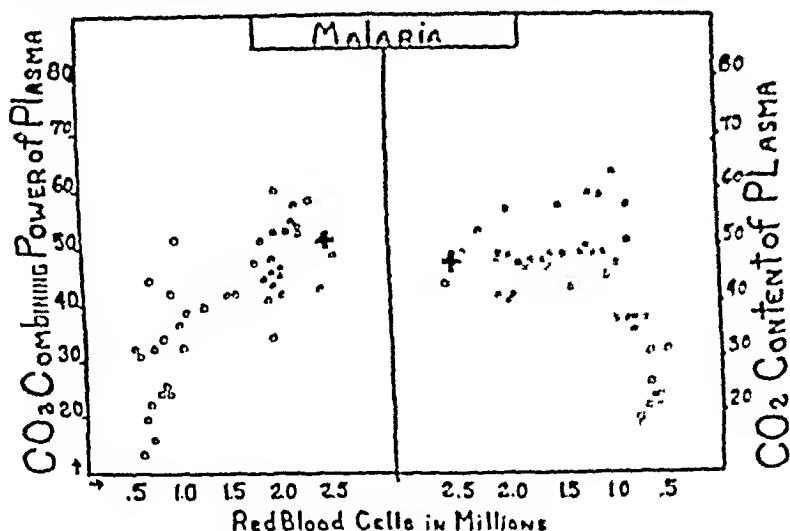


FIG. 1. The average CO_2 combining power of the plasma from 12 normal ducks is 51.4 volumes per cent and the average CO_2 content of the plasma of 21 normal ducks is 46.9 volumes per cent. These values are indicated by +. The CO_2 combining power of the plasma in 61 ducks and the CO_2 content of the plasma of 44 ducks with malaria are indicated in the graph. Note the development of the acidosis with the anemia. The CO_2 content of the plasma remains high until the number of red cells decrease below one million.

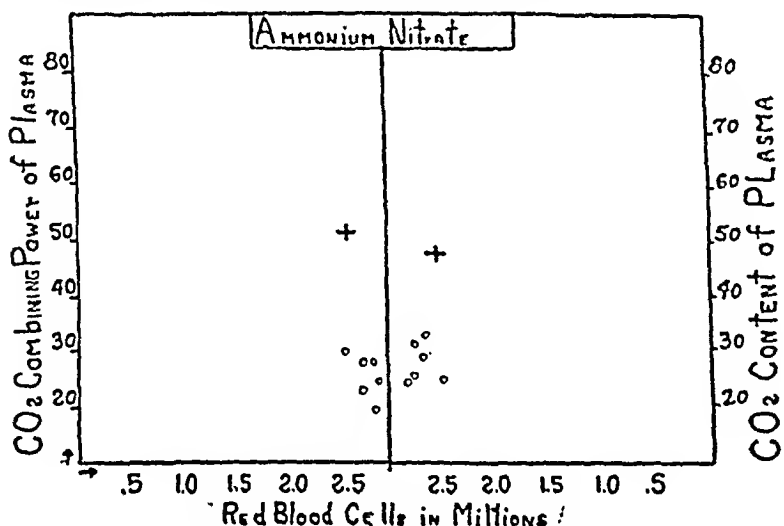


FIG. 2. The CO_2 combining power and CO_2 content of plasma from six ducks given large doses of ammonium nitrate. + indicates the control values. A severe acidosis occurs with no anemia.

In an attempt to evaluate these chemical changes occurring in the malarial infected ducks, six birds were given orally large amounts of ammonium nitrate. The carbon dioxide content and the combining power of the plasma are shown in fig. 2. There occurs a severe acidosis with little variation in the number of

red blood cells within the peripheral blood. Accompanying this acidosis there is a marked decrease in the carbon dioxide content of the plasma.

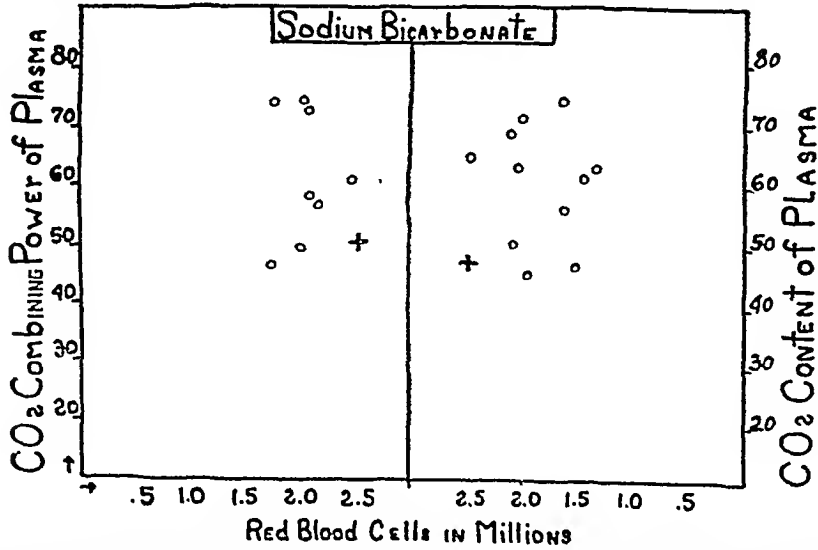


FIG. 3. The CO_2 combining power of the plasma of 8 ducks and the CO_2 content of plasma from 11 ducks given sodium bicarbonate. + indicates the control values. A severe alkalosis occurs with a slight anemia.

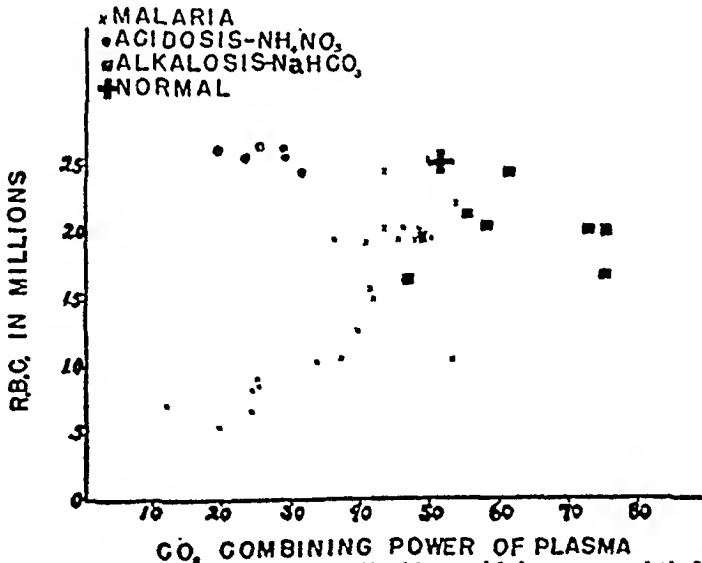


FIG. 4. The composite data of the carbon dioxide combining power of the plasma of the group of birds with malaria, those given ammonium nitrate and sodium bicarbonate, and the controls.

The carbon dioxide combining power of the plasma of 8 ducks given orally large amounts of sodium bicarbonate and the carbon dioxide content of the plasma of 11 ducks similarly treated are shown in figure 3. These data show that with the development of the alkalosis there is only a slight decrease in the number of red blood cells. There is a marked increase in the degree of retention of carbon dioxide in these ducks. Figure 4 shows the composite data of the

carbon dioxide combining power of the plasma in relation to the number of red blood cells in the peripheral blood in normal birds, the group of ducks with malaria, those given sodium bicarbonate and those given ammonium nitrate. Figure 5 shows the carbon dioxide content of the plasma on the corresponding group of ducks. From these data one may conclude that the acidosis which occurs in malaria may not result entirely from the anemia since ducks with an acidosis resulting from ammonium nitrate show no anemia. The production of acid by-products by the parasites may be contributory.

The carbon dioxide in the plasma of ducks infected with *P. lophurae* remains at approximately the same level until the anemia reaches less than one million cells where upon it rapidly decreases. The number of parasites, however, rapidly increase during the time in which the carbon dioxide remains constant.

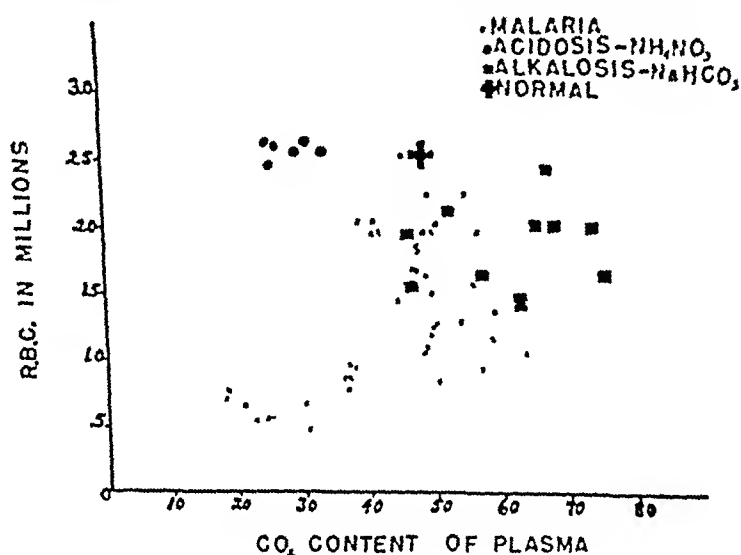


Fig. 5

To determine the relation of the total number of red blood cells to the carbon dioxide in the plasma the carbon dioxide content of the plasma was divided by the number of red blood cells in millions. This factor was then plotted against the total number of red blood cells. A similar factor was obtained for the carbon dioxide content of the plasma in ducks given sodium bicarbonate and ammonium nitrate. The results of these observations are shown in figure 6. These data show that the results of the observations on the carbon dioxide content of the plasma in ducks with malaria and those given sodium bicarbonate follow an identical course. With the decrease in the number of red cells the carbon dioxide content of the plasma increases. Ducks made acidotic with ammonium nitrate on the contrary show a marked decrease in the amount of carbon dioxide in the plasma with no variation in the number of red cells. When the figure obtained by dividing the carbon dioxide content of the plasma by the number of red cells in millions is plotted against the number of parasitized red

blood cells we find that the carbon dioxide content of the plasma increases with the increase in the number of parasitized cells. The greatest increase is observed

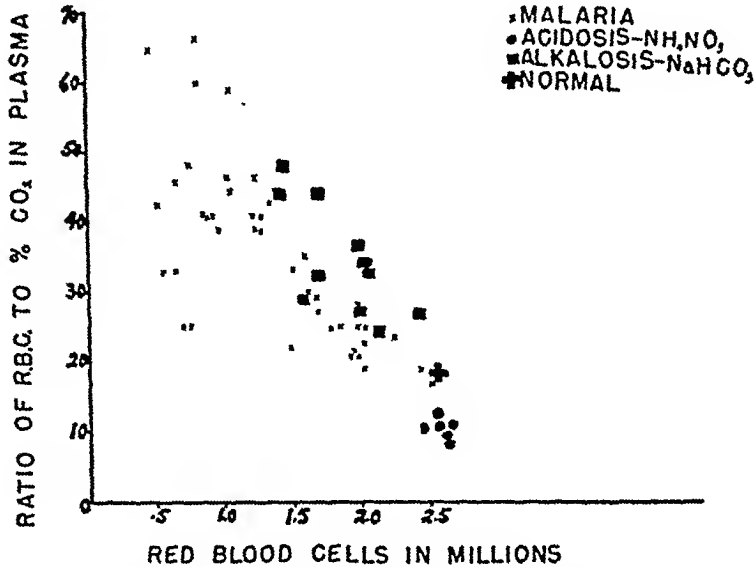


FIG. 6. The figure obtained by dividing the CO₂ content of the plasma by the number of red blood cells in millions is plotted against the total number of erythrocytes for the ducks with malaria, those given sodium bicarbonate and ammonium nitrate, and the control group. Note that the distribution of the points for the ducks with malaria parallel those of the birds given sodium bicarbonate.

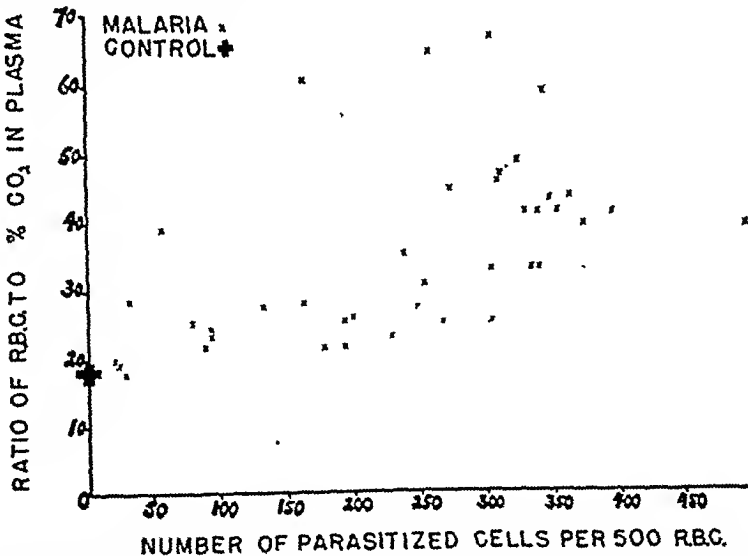


FIG. 7. The figure obtained by dividing the CO₂ content of the plasma by the number of red blood cells in millions is plotted against the number of parasitized cells per 500 red blood cells. Note that this value increases with the increase in the number of parasitized cells.

when the number of parasitized cells is 250 or more per 500 red blood cells (fig. 7). This increased amount of carbon dioxide within the plasma may be

sufficient to either injure or kill some of the plasmodia. This opinion is supported by the *in vitro* observation that *P. lophurae* are killed when carbon dioxide is bubbled through the inoculum (15).

In conclusion it may be stated that factors other than immunologic may be significant in producing the death of *P. lophurae* in ducks. Among these may be mentioned the large number of young erythrocytes that are present at the time of the peak of the parasitemia. These young cells carry only a small amount of hemoglobin and oxygen. These plasmodia prefer adult erythrocytes to these young cells. The retention in the blood of metabolic products produced by the plasmodia may injure the parasites. There is a shift in the pK of the whole blood during the course of the malarial infection from 6.2 to 6.05 (19). This, of course, means a failure of the bicarbonate buffer system. This might be interpreted to mean that a higher CO₂ tension in the malarial blood exist at some time during the disease.

SUMMARY

Some of the factors that produce a diminution in the number of parasites in *P. lophurae* infected ducks have been discussed. It has been shown that an increase in the carbon dioxide content of the plasma occurs with the increase in the degree of parasitemia. It appears that carbon dioxide may be a very important factor in producing the death of plasmodia following the time of the peak of the parasitemia. Furthermore in support of the opinion that factors other than immunologic play a significant role in regard to the degree of parasitemia it has been shown elsewhere that the number of parasites may be prevented from decreasing by the intravenous injection of large numbers of red blood cells. The number of parasites is greater in polycythemic than normal ducks. These plasmodia also prefer adult erythrocytes to young red blood cells. Phagocytosis occurs in ducks infected with *P. lophurae* and no doubt certain immunological substances are produced, however, these alone do not appear to be adequate to account for the phenomenon of the rapid decrease in the degree of parasitemia following the peak of infection.

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PLAGUE: A SURVEY OF RECENT DEVELOPMENTS IN THE PREVENTION AND TREATMENT OF THE DISEASE¹

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Since the beginning of the Christian era, plague has been one of the principal infectious diseases which affect the human population in epidemics. Much was learned concerning these epidemic scourges and even primitive peoples made observations enabling them to forecast the appearance of the disease in epidemic proportions. Modern concepts of the etiological nature and pathogenesis of the disease date from 1894 when two independent workers discovered almost simultaneously that the causative agent was a bacterium, subsequently classified as *Pasteurella pestis*. Following the epidemic of 1894, which had its origin in south-western China, plague attained world-wide dissemination and every civilized country was compelled to take steps to curb the disease. Since then much has been learned concerning the malady, its epidemiology, treatment and control. However, the explosive nature and trigger mechanism which sets off an epidemic still require elucidation. For those reasons plague remains a potential danger even in the countries of the western hemisphere where methods of control are most efficiently organized.

The global nature of World War II is manifest by the increased awareness of plague among the belligerent nations of this conflict. This awareness is especially evident by the great number of medical publications on all phases of the disease. The monumental work by Wu and his co-workers (1) of the National Quarantine Service in Shanghai, China, remains the standard reference on plague. Significant developments in the treatment and prevention of human plague have occurred subsequently which deserve wide publicity in order to make possible clinical and epidemiological application of these principles. In addition, a new awareness of the economic and sociologic implications is becoming apparent. Hence the problem has become not exclusively a medical one but must be the responsibility of some international organization, of which medicine is only one facet. These introductory remarks gain import by the timeliness of local conditions throughout the world. The relaxation of regulations entailed by the exigencies of war, the transfer of populations following the cessation of hostilities, the stimulus to travel and migration engendered by the war and the imponderable effect of social and political ferment among colonial and semi-colonial peoples are factors of compelling urgency in an early consideration of the plague problem today.

Laboratory research with trials on animals and clinical tests on humans have proved the value of new biological and chemical products. Similarly, chemistry has provided new weapons to approach the disease from a mechanical standpoint, by breaking the chain of transmission of the causative agent. The amelioration

¹ From Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y.

of social conditions, especially among the lower economic groups, is also an important factor in the control of plague. Favorable results along one or more of these lines have been reported from many of the plague-conscious areas of the world, especially from Argentina, China, Egypt, French North Africa, India, Java, Kenya, Peru, the Soviet Union, the Union of South Africa and the United States. Several studies are worthy of especial reference. Dr. K. F. Meyer summarized very succinctly the history of plague and progress made in the control of the disease before the New York Academy of Sciences (2) in 1946. More recently, Dr. John Gordon and Dr. Philip Knies (3) of Harvard University School of Public Health published a review of the various types of work and organization required for the control of plague. About two years ago, Lieutenant R. F. Platzer, U.S.N.R. (4) published an evaluation of the various products available in 1943 in the treatment of plague. Subsequently, numerous other publications, usually narrower in scope, have appeared and provide the basis for this survey.

PREVENTION

The first effective procedure employed in the control of plague was *quarantine*; even today, quarantine remains an important part of our prophylactic armamentarium. Corollary to quarantine has been *isolation* as a method of preventing the spread of the disease. Both of these measures have their limitations but should become integral parts of every anti-plague program. In order to make these two measures effective and popular—in the last analysis, public health measures depend on popular support for their effectiveness—a well-organized, alert *medical intelligence service* (3) must be set up; this entails the training of special personnel in methods of diagnosis as well as epidemiology. Such personnel can be stationed at key points and will be ready to act if their peculiar interpretation of medical, military or civilian reports is suspicious of the diagnosis of plague in man or animals.

Theoretically, *animal reservoirs* are vulnerable links in the conquest of disease. Consequently, the finger of suspicion has long been pointed at the rat in any plague-control program. The introduction of raticidal chemicals and drugs has therefore raised hopes that plague may be abolished. Macchiavello (5) reported the extensive use of "1080," a fluorine-containing preparation, in Peru and considered the chemical to be quite effective in reducing the rat population. The discovery of a raticidal agent which is non-toxic to other small animals or to humans might be a welcome addition to the anti-plague armamentarium. However attractive the rat-suppressive thesis might be in the control of plague, such programs are subject to technical difficulties which usually defeat the main purpose. Gordon (3) feels that a "direct attack on the flea instead of indirectly against the animal host. . . would unquestionably be a distinct advance." The experience of workers in the western United States would lead one to believe that elimination of rats entirely would not be the complete answer to the plague problem; the intermediate vector is apparently able to adapt its parasitic life to other rodents or even to certain avian hosts. *Improvements* in economic conditions, so that man and rat would not be co-habitants, would be a more ideal, although more expensive, approach to this angle of the plague problem.

In 1941, Dr. I. J. Joff published an extensive work (6) in the U.S.S.R. on the epidemiological importance of fleas in human plague. Subsequently, both Gordon (3) and Meyer (2) recorded their opinions that the most practical approach to plague control, once an epidemic is imminent or in progress, is the *destruction of the flea*. The pulicidal efficacy of "DDT" has been confirmed invariably by workers in plague. It is inexpensive, relatively non-toxic to humans and can be utilized efficiently by untrained workers. Other pulicidal agents have been used but DDT is preferred at present because of its relative lack of toxicity for humans and its lack of specificity for insects and other parasitic animals. In fact, it is not inconceivable that this or a similar agent might be the means of eradicating plague even from its animal reservoirs.

Effective repellents have not been eminently satisfactory. At present, the introduction of methods of impregnating DDT into clothing has diminished somewhat the need for flea-repellents in anti-plague programs.

A fourth preventive method of attack on the plague problem is directed *against the organism* itself. Conventionally, this is effected by active or passive immunization; still another means of attacking the organism has been afforded by the prophylactic exhibition of the sulfonamides. Pollitzer (7) reported apparent success from China in preventing plague among intimate contacts by the administration of sulfadiazine or sulfathiazole daily. Lewis, Buehler and Young (8) recorded a similar experience in Oran when a threatened epidemic of pneumonic plague was brought under control by the administration of 3 grams of sulfathiazole daily to 85 contacts; only one person in this group contracted the disease. Meyer (2) records the successful use of sulfadiazine in preventing the disease among laboratory workers following accidents in the laboratory. Similarly, there has been a recent report from China which tended to show the prophylactic value of sulfadiazine in preventing secondary cases among the contacts of a patient with pulmonic plague (9). Subsequent investigations in the laboratory as well as in clinical practice have tended to show that sulfadiazine is superior to sulfathiazole in the battle against plague. While there has been no field trial of the drug on a large scale as a prophylactic, the mass of laboratory data and the results of prophylaxis against certain other susceptible organisms are sufficient to recommend sulfadiazine as the effective prophylactic worthy of extensive field trials. Moreover, in bubonic plague there is no danger of developing drug-resistant strains of the organism by prophylactic administration of the sulfonamides.

Active immunization in plague has been recommended for a number of years. The multivalence of the *P. pestis* organism and the lack of correlation of antibody titrations in test animals have made evaluations of plague vaccines most difficult. It is the opinion of Meyer and his associates (10) that it is "advisable at the present time to use vaccines containing all of the antigens of *P. pestis* for prophylaxis of plague in man." The preparation of a potent vaccine which would not cause severe reactions in humans has been difficult of preparation. At the Haffkine Institute of Bombay, a potent vaccine of killed virulent organisms has been prepared. However, Simeons and Chhatre (11) in analysing 1000 cases of bubonic plague treated in India, doubted that the inoculation of a single dose of the vac-

cine had any protective action. Likewise, a similar vaccine was considered to be without effect in protecting humans in Java. The United States Army was immunized with a formalin-killed agar-grown suspension of virulent plague bacilli in carbolized saline. An immunizing dose consisted of 2 injections of 0.5 and 1.0 ml. at intervals of 7-10 days; recall inoculations were given every six months. Such a military population as the American Army is scarcely ideal for the evaluation of the efficacy of a plague vaccine; but the fact that not a single infection with *P. pestis* occurred in this group (3) despite probable exposure in many areas is suggestive that the vaccine may have had a preventive effect. Wayson and his colleagues (12) of the United States Public Health Service have recently reported the superiority of an alcohol-precipitated vaccine over other vaccines, including the one used by the United States Army. They further point out that the degree of protection bears some relation to the size of the dose of vaccine and that doses of relatively large numbers of bacteria are necessary for maximal immunity. Grasset (13) noted, too, that large numbers of organisms were required for an effective vaccine. The latter worker employed a single injection of one billion live organisms of an avirulent strain for the immunization of adults in South Africa; the efficacy of the vaccine is attested by the occurrence of only 15 cases of plague, with 7 deaths, among 24,000 vaccinated persons during 14 epidemics. He observed that protection began 5 days after vaccination and was complete in 10 days.

A pessimistic note, not without some truth, is sounded by M. B. Bayly (14) who thinks that vaccine diverts attention from the removal of the fundamental cause of the disease. He noted further that plague "disappeared as the people became more cleanly in their habits. Cleanliness is the master word." Unfortunately, vaccines must still be advocated until the under-privileged masses, especially in the Orient, learn the virtues of cleanliness!

The establishment of passive immunity by the prophylactic inoculation of anti-plague serum has been effected and the prophylactic value of serum demonstrated in humans as well as animals. The short duration of passive immunity is objectionable but unavoidable. The effective use of sulfonamides and vaccines as prophylactics makes consideration of serum as a prophylactic both unnecessary and impractical.

TREATMENT

The effective treatment of plague has been a development of recent years. Even now, the great variation in mortality of the various epidemics and variations in mortality from place to place during the same epidemic requires much caution in evaluating results.

The value of serum as a specific therapeutic agent in plague is not lacking in the medical literature but technical difficulties in the production of a potent serum have prevented its popular use. Recently, Bhatnagar and Shrivastava (15) in Bombay have made a very pretty contribution to the studies of cellular immunity in experimental plague infections. And Korobkova (16) in Moscow confirmed the specific action of anti-plague serum. Platzer (4) points out in his

review that there has been considerable difficulty in the development of a potent serum which could be administered to humans without causing alarming reactions. In the United States, a serum has been produced by immunizing rabbits (17) with killed avirulent organisms; while this refined serum is pyrogen-free and considerably more potent than previous sera, its use in humans has not yet been reported. Girard (18) feels that serum has a place, especially in the treatment of the particularly toxic cases of plague. The objections to all sera, namely, expense and necessity for parenteral administration, applies to anti-plague serum, and may conceivably be its limiting factors.

Bacteriophage had its advocates following its introduction by d'Herelle. It has not been used in a large controlled series of cases but Sorel (4), in reviewing its use in the French Colonies, concluded that its clinical benefits were not apparent. Workers in American laboratories are of a similar opinion.

The sulfonamides constitute our most effective therapeutic agent in plague. While all of the sulfonamides have been shown to have curative effects in isolated cases, the majority of workers in the treatment of plague feel that sulfadiazine is the sulfonamide of choice at present (11, 19-26). Yet, not a single sulfonamide has been subjected to a large-scale well-controlled therapeutic trial in the field under epidemic conditions.

Suggestive confirmation of the curative effect of the sulfonamides in human infections is accumulating. The dramatic recovery of a physician with primary pulmonary plague, reported in detail by Minter (22) is presumptive evidence of the efficacy of sulfadiazine since spontaneous recoveries from the pulmonary form of plague have been rare before the sulfonamide era; the influence of previous vaccination in this case cannot be evaluated. More recently, two French physicians (24) have reported three cases of pulmonary plague recovering after treatment with the sulfonamides. In view of the almost universally fatal nature of this form of the disease prior to the introduction of sulfonamides, it is not unreasonable to believe that certain of these drugs exercise an unequivocally beneficial effect on the disease.

Subsequently other reports have been published from most of the epidemic areas. Wagle (25) confirmed the value of sulfadiazine and sulfathiazole which reduced the case fatality rate to 20 and 37 per cent respectively whereas with "other" treatment the case fatality rate in the same areas was in excess of 90 per cent. Sokhey and Wagle (19) had an experience quite similar in degree and differing only in detail; they preferred sulfadiazine because of its lesser toxicity. Simeons and Chhatre (11) after treating 1000 cases of plague with sulfathiazole or sulfadiazine felt that sulfadiazine was the superior drug. A report from Fukien, China (20) adds to the mounting evidence that sulfadiazine is the drug of choice in the treatment of plague. A preliminary report from UNRRA workers in Manchuria underlines the value of this agent in dealing with the epidemic disease (27).

The relatively recent introduction of the antibiotic treatment of infectious diseases has raised the hope that some more effective agent against plague might be found. Streptomycin has been shown by Wayson and McMahon (28) to be effective in curing plague infections in guinea pigs. The human clinical applica-

tion of this finding awaits confirmation. At present, streptomycin shares in some respects the objections to serum, namely, expense and necessity of parenteral inoculation.

Dr. C. K. Chu in discussing the current plague situation in China (9) reported a unique incident which suggests a more hopeful prognosis for pulmonic plague victims. A physician developed plague pneumonitis while engaged in transferring virulent cultures in the laboratory. Sulfadiazine therapy was instituted in full doses on the second day of symptoms after the diagnosis had been confirmed culturally. On the fourth day and for several days thereafter, streptomycin was also administered. The patient made an uneventful and complete recovery, thus bearing out the prediction implied in the experimental work of Wayson and McMahon (28).

Thus at the present time at least three effective agents are available in the treatment of plague in humans: anti-plague serum, sulfadiazine (and certain other sulfonamides) and streptomycin. The possibility of combination with resultant potentiation of therapeutic action requires clinical trial. Likewise, there is no reason to think that the last chapter has been written in either chemotherapeutic or antibiotic therapy of bacterial disease.

CONCLUSIONS

1. Plague as an epidemic disease is amenable to control.
2. An anti-plague program should include (a) quarantine and isolation (b) control of rodent populations (c) emphasis on pulicidal measures and (d) prophylaxis of the individual contacts.
3. Effective therapeutic agents are now available.
4. An effective program to prevent epidemics and to control plague as a world disease must embrace measures designed to alter economic and social conditions as well as medical and epidemiological measures.

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METHOD FOR TESTING OINTMENTS AND FABRICS TO DETERMINE THEIR EFFECTIVENESS AS BARRIERS TO SCHISTOSOME CERCARIAE¹

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As the war moved from the islands of the Pacific toward Japan and the mainland of Asia, schistosomiasis became a disease of military importance. The need of having a broader knowledge of blood worm infection in the field, especially as related to Americans whose duty carried them through endemic areas, was emphasized especially in late 1944 when, during the invasion and recapture of the Philippine Islands, a considerable number of military personnel contracted the disease. Research investigation groups were sent into the field to evaluate the schistosomiasis problem and to study its epidemiology, its clinical aspects, its treatment, etc. One aspect of the problem was the study of methods by which personnel could avoid or be given protection against the disease. Previously warring peoples have never been faced with this disease to such an extent as to warrant extensive studies toward the protection of man from infection. Therefore in World War II investigators were confronted with a problem which, in certain aspects, had been relatively unexplored. It has been attacked from several angles:

- a. Destruction of the schistosome-bearing snails which to date has been only moderately successful.
- b. Temporary elimination of cercariae by cercaricides.
- c. Immediate protection to personnel by use of uniforms made of protecting fabrics, proper donning of uniforms and by application of barriers and so-called "repellents" (greases, ointments, etc.) to the skin.

Several laboratory methods (1, 2, 3, 4, 5, 6, 7) have been described for testing fabrics and ointments. In employing these methods, at least in those using a host, it has been necessary to keep the test animals for 15 to 40 days, dissect them and make a parasite count to determine the degree of protection afforded by the materials of concern. Therefore, it was highly desirable to develop a method which would yield accurate results in the shortest possible time. An improved method is described in this report.

To those familiar with schistosomiasis in lower animals (8) and man (9, 10) the appearance of a rash or dermatitis following an attack by schistosome cercariae is well known. Assuming that some laboratory animals might show a similar reaction to cercarial penetration, exploratory tests were run to determine

¹ The opinions or conclusions contained in this paper are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

The authors gratefully acknowledge the suggestions and help given by Lieutenant L. A. Jachowski, Jr., HS, USNR and Lieutenant F. H. Hyslop, HW, USNR.

the nature of skin reaction in different animals and to determine the hosts which manifest the most prominent dermatitis. Accordingly rabbits, cats, dogs, hamsters, guinea pigs, rats and mice were exposed to the cercariae of *Schistosomatum douthitti* (Cort 1914) (a schistosome which is non-pathogenic to humans but which may cause "swimmers' itch" in man) and *Schistosoma mansoni* Sambon 1907. *S. douthitti* gave the better dermal reaction whereas the cercariae of *S. mansoni* seldom incited a noticeable dermatitis.

In the lower mammals in general, as in man, the degree of reaction varies considerably with the individual, but in the rabbit the sites of cercarial penetration (by *S. douthitti*) have a fairly uniform appearance and can be used as a quantitative index of penetration. The rather uniform reaction is a desirable feature upon which the described test method depends.

Since the cercariae of both *S. douthitti* and *S. mansoni* penetrate laboratory hosts (hamsters and white mice) with equal facility, as determined by subsequent recovery of adult parasites on dissection, it may be assumed that the penetration counts for *S. douthitti* also will apply to *S. mansoni*. Therefore by the use of selected white rabbits as test animals and the dermatitis-producing cercariae of *S. douthitti* as invading agents the effectiveness of various materials against penetration by the human pathogen *S. mansoni* may be evaluated.

MATERIALS AND PRELIMINARY PROCEDURES

Parasites and hosts. The larvae of *S. douthitti* and *S. mansoni* were maintained in the laboratory in the intermediate snail hosts, *Lymnaea stagnalis* and *Australorbis glabratus* respectively. The adult worms were carried in white mice and in golden hamsters.

Procedure for selecting the ideal test animal.—All rabbits, cats, dogs, hamsters, guinea pigs, rats and mice exposed for 30 minutes, in the manner described, to a suspension of *S. douthitti* cercariae showed some degree of dermatitis. In most instances, however, white rabbits (6 months of age) gave the best skin reactions and in the majority of exposures the respective sites of inflammation resulting from cercarial penetration could be detected easily. Certain test rabbits demonstrated especially prominent reaction sites. These sensitive-skin animals were segregated. When used repeatedly they showed a constant, better than average reaction. The cercariae of *S. mansoni* seldom produced obvious signs of dermal reaction and on no occasion, even in the more susceptible rabbits, did the dermatitis equal that produced by *S. douthitti*.

Comparison of the infective potentialities of the cercariae of S. douthitti and S. mansoni. The logical criticism of the use of a test method in which a non-pathogenic organism is employed to evaluate the infective or invasive potentialities of a pathogenic organism, is that the results obtained from the former (*i.e.*, cercariae of *S. douthitti*) may not be applicable in judging the infecting ability of the latter (*i.e.*, cercariae of *S. mansoni*). When mice or hamsters, both satisfactory hosts for the two parasites (11), were subjected to a given number of viable cercariae it was demonstrated that a certain percentage of that number would be found as adult schistosomes upon dissection of the host several weeks after infection. Since the actual recovery of worms from a mouse or ham-

ster exposed to a counted number of cercariae of *S. douthitti* approximates that found for *S. mansoni* it may be assumed that the invasive potentialities of the two parasites are similar.

The ability of the two different species to pass barriers should likewise be similar. This assumption was validated by infection experiments employing shaved mice and hamsters. After removal of the hair, a uniform covering of test ointment (petroleum vasoline or heavy motor oil) which was known by experiment to be only a fair or moderately effective barrier, was applied to belly

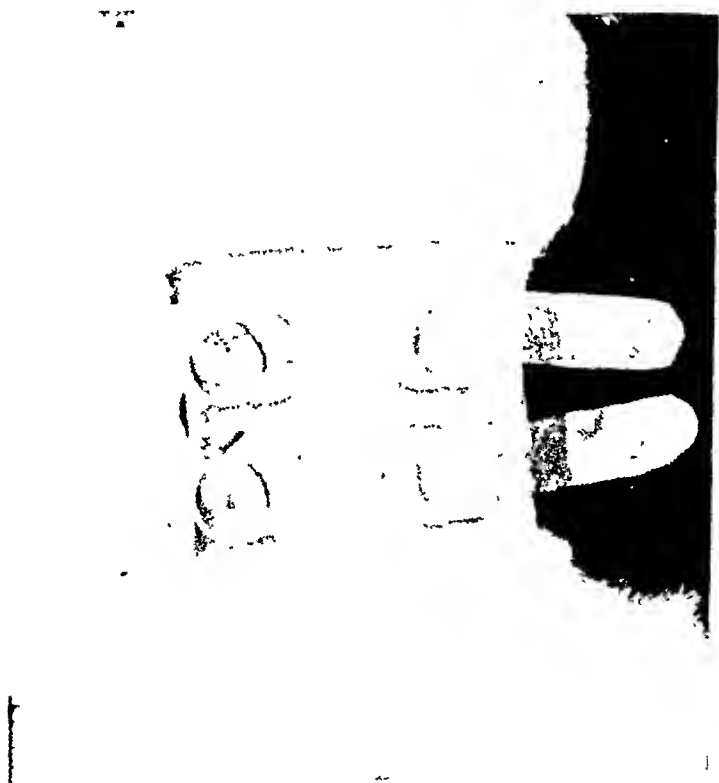


FIG. 1. OINTMENT BELT ON RABBIT

skin. Hosts were exposed to suspensions of 25 cercariae of either *S. douthitti* or *S. mansoni* as described above. At autopsy, 3 to 4 weeks after exposure, the number of worms recovered from *S. douthitti* and *S. mansoni* exposed animals fell within a reasonable and acceptable range. Usually 3 to 8 worms were recovered.

It can be assumed then, that, the number of sites of inflammation produced by cercariae of *S. douthitti* passing through a barrier and invading the skin of test animals is comparable to the number of *S. mansoni* cercariae passing a barrier but not producing a readable dermatitis.

THE TEST PROCEDURE

Having determined the combination of parasite and test host which would give the most satisfactory and most reliable skin reaction it became necessary to devise a belt and the method for test procedure.

Belts for holding cercarial suspension containers on test rabbits. For practical purposes, belts were made for holding several test chambers. Considerable inconvenience was experienced in preliminary tests in attempting to hold several or at times even a single container in position. Two types of cercarial suspension containers were necessary for testing ointments and fabrics.

a. Belt with ointment test containers. Four pieces of glass tubing one inch in diameter and one and one-half inches long, which served as the test containers, were fitted into a belt made from a 3 by 14 inch strip of automobile inner tube rubber (fig. 1). The rubber was cut so as to leave two strips or tongues one inch wide and about four inches long on each end. These strips allowed for the at-



FIG. 2. FABRIC TEST BELT ON RABBIT

tachment of buckles used to pull the belt tightly against the host. A one-half inch length of rubber tubing, about one-half of the diameter of test container, was slipped over the end of the glass tube and was in contact with the host's skin. This rubber when flush with or protruding slightly beyond the end of glass tubing served as a washer to prevent leakage, as well as a resistant collar to hold the tube more securely against the under side of the rubber belt. The tubes were fitted into the belt by forcing their opposite ends through holes about one third the diameter of the tubes. A test belt of this size placed on the rabbit's belly provided four chambers each exposing an area of skin for testing. Three different ointments could be tested simultaneously with the fourth area designated as the control.

b. Belt with fabric test containers. With minor modification the same belt as described above was used for testing clothing and fabrics (fig. 2). The

sample tested was soaked in water, stretched over one end of a one and one-half inch length of glass tubing (one inch in diameter) and held securely in position by a rubber tubing one-half inch long slipped over the cloth. This unit was inserted into a piece of glass tubing one and one-quarter inches in diameter. The inner glass tube with the test sample and a rubber band at one end to serve as a bushing, was fitted tightly within the larger tube and prevented leakage of the cercarial suspension. Another rubber band was placed on the end of the larger outer tube and contacted the rabbit. Four of these double tube units were thrust through perforations in the rubber belt as mentioned above. The belt (or two belts if desired) was buckled and drawn tightly around the rabbit until the skin bulged into the test containers. Adjustment was made so that the test cloth over the lower end of the inner glass tube came to rest 1 to 3 mm. above the surface of the skin. The test samples were soaked in water several minutes before the beginning of the test. Space between the rabbit's skin and the test cloth was filled with water by means of a small gage hypodermic needle inserted along the side of the rubber bushing between the inner and outer tubes. A counted number of cercariae in water was placed in containers above the sample. To reach the skin cercariae had to pass through the test barrier.

Application of test barriers. Various ointments and greases were tested to determine their effectiveness as barriers to schistosome cercariae. These were applied to the test areas by either of two methods. The belt with four test containers was held with some force against the belly of the anesthetized rabbit for a minute or so to make imprints of the test chambers of the skin. After removal of the belt circular areas within the impression were covered uniformly with ointment. Viscous ointments were spread on the skin by hand and less viscous preparations were applied with either the finger or a cotton swab. The belt was then placed on the belly skin. With the second method of application the ointment was spread on the skin of the test host by sticking a cotton swab through the open ends of the containers after the belt was tightened and in position. A counted number of cercariae was pipetted into the test chambers.

Clothing samples were handled as described above. Little or no trouble was experienced in changing and testing the different samples which varied considerably in weave, thickness, etc.

In order to eliminate error in counting penetration sites or in determining areas of exposure at reading time, each area was circled and numbered with either picric acid or India ink.

Periods of exposure of cercarial suspension to skin of test rabbits. Exposure time in testing ointments and fabrics differed because much more time is required for a cercaria to work its way through fabrics. Experiments indicated that 30 minutes is adequate time to allow cercariae to invade a rabbit's skin with or without a penetrable ointment barrier. Small numbers of cercariae were used. If more than 25 cercariae were used the resulting areas of inflammation around the penetration sites often overlapped and made accurate counting difficult. Further experiments demonstrated that only a small percentage of a cercarial population could pass clothing barriers. Therefore in testing such barriers the number of cercariae was increased to 50 and the exposure extended to 60 minutes.

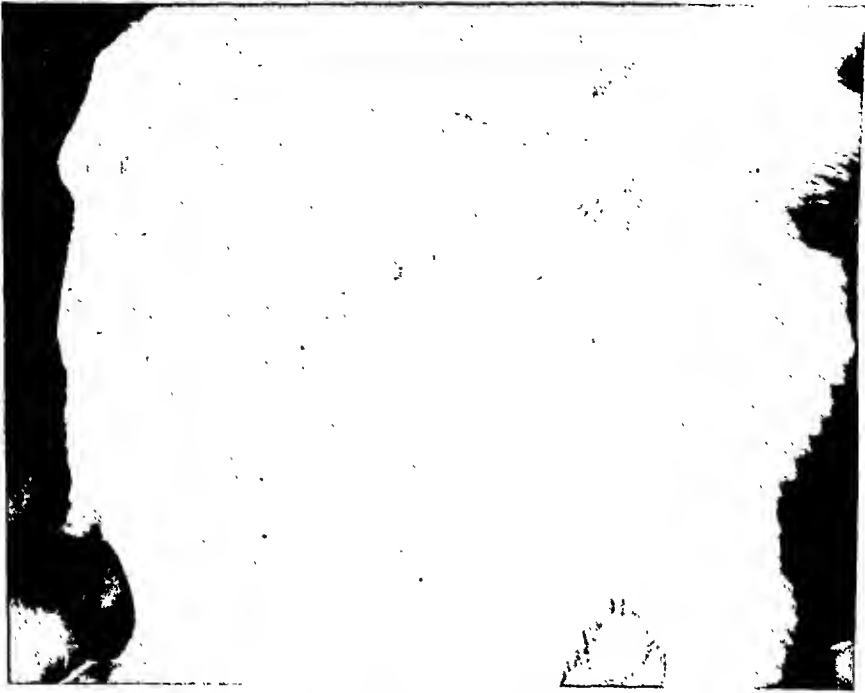


FIG. 3. CERCARIAL PENETRATION SITES EIGHT HOURS AFTER EXPOSURE



FIG. 4. CERCARIAL PENETRATION SITES 24 HOURS AFTER EXPOSURE

Examination of rabbits after test. Rabbits were examined at four to eight-hour intervals and up to 48 hours to count the inflammation spots in the exposed areas

indicating the number of cercariae of *S. douthilli* which had penetrated the barrier. On occasion the dermatitis reached its maximum 8 to 12 hours after the test. As a rule the penetration sites were hardly visible and often difficult to detect at the end of the first or second four-hour interval (fig. 3) but they were easily counted after inflammation had progressed for 20 to 24 hours (fig. 4).

SUMMARY AND CONCLUSIONS

Ointments and clothing samples can be given a general screening to determine their value as barriers in protecting man from infection by schistosome cercariae. The method depends upon the development of a rash with local inflammatory reaction at the site of cercarial penetration in mammalian skin and upon the count of these penetration sites about 24 hours after exposure of the skin test area to a counted number of cercariae. Test samples can be handled with relative ease and results obtained quickly.

This method of testing schistosome cercarial barriers appears to be an improvement over previously described technics because:

- a. The number of test animals is held to a minimum; test rabbits need not be sacrificed and may be used over and over again.
- b. There is no 4 to 6 weeks' period of delay between testing (*i.e.*, time of host infection) and time of autopsy, as is necessary with other technics.
- c. There is no need for time-consuming examination of autopsied hosts to determine the presence or absence of adult worms.

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VISCERAL LEISHMANIASIS COMPLICATED BY SEVERE ANEMIA—IMPROVEMENT FOLLOWING SPLENECTOMY

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Visceral leishmaniasis in members of the armed forces of the United States has been reported occasionally in the past two years (1, 2). The purpose of this article is to report the therapy and the results of therapy in four cases of this disease, and to discuss in detail the fourth case, which differs considerably from the others both in the degree of the clinical manifestations and in the response to therapy. Whereas the anemia in kala azar does not usually reach a hemoglobin value of much below 40 per cent of normal, this last patient needed 102 transfusions, each of approximately 500 cc. of whole blood, to maintain a hemoglobin level of from 20 to 40 per cent. In most instances kala azar responds dramatically to adequate doses of the pentavalent antimony compounds, neostibosan and stibanose, or to the non-antimony-containing aromatic diamidino compound, 4:4' diamidino stilbene. This case, however, apparently failed to respond to what are usually considered adequate doses of these drugs and finally did respond only to very massive doses of diamidino stilbene. Another point of interest is that splenectomy, definitely contraindicated in the usual case of visceral leishmaniasis, in this one patient appears to have been a life-saving measure.

Visceral leishmaniasis is a disease characterized by invasion of the reticulo-endothelial system by a protozoan parasite, *Leishmania donovani*. It had been observed in India as kala azar or DumDum fever, and in the Mediterranean as pones, but the cause was unknown until 1903, when simultaneous investigations by Leishman at Netley, England, and Donovan in Madras, India, demonstrated the parasite in splenic preparations. The disease, presumably carried by sandflies of the genus *Phlebotomus*, is now known to be widespread. Outbreaks of the disease have been noted in India, China, Southern Russia, the Mediterranean littoral, various areas bordering on the Sudan, and in Brazil. It is probable that all cases reported in this particular series became infected either in Sicily or Tunisia.

It is unnecessary here to discuss in detail the differential diagnosis, diagnostic methods, or the history of the development of the various compounds used in the treatment of kala azar. This may be found in a previous paper (1) or in any standard textbook of tropical medicine (3), (4). Suffice it to say that modern methods of therapy have reduced a mortality quoted by various sources as averaging from 80 to 97 per cent to a present rate of from 0 to 10 per cent.

REPORT OF CASES

Four patients with visceral leishmaniasis have been treated by the senior author in the past two years. Of these, cases 1, 2, and 3 have already been

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reported in a previous paper (1) and their mention here is only in the nature of a follow-up on the results of therapy. All were in excellent health when last heard from 29 months after therapy and must be considered cured. The fourth case, however, deserves closer attention and it is to this patient that the major part of the paper will be devoted.

This 23 year old negro soldier landed in North Africa on 1 January 1943 and spent the next 14 months in air field construction work in or near Algiers and Tunis. He was perfectly well until the early part of February 1944, when he noted he tired more easily and was losing weight. Shortly afterwards chills and fever began, and the patient was sent to a station hospital on 27 February 1944. Hematologic studies showed 1,900,000 red cells, 50 per cent hemoglobin and 1750 white cells with 32 per cent polymorphonuclear leukocytes. The hematocrit was 18 and the sedimentation rate was 18 mm per hour. The urine was normal.

Quinine, penicillin, sulfadiazine, and transfusions were administered with little benefit to the patient, and five weeks later he was transferred to a general hospital. On physical examination the spleen extended three fingers breadths below the left costal margin. The x-rays of chest and long bones were negative. No sickling was noted on preparations of anoxic blood, the osmotic fragility was normal, and the icteric index was 5. On 14 April a splenic puncture was done and *Leishmania donovani* were found in smears stained with Giemsa's stain. The total protein was 7.8 grams, with the albumin 3.1 grams and the globulin 4.7 grams. The formol gel test was positive. Hematologic studies were approximately the same as previously reported.

Neostibosan, anthiomaline, and solustibosan were given without effect and the patient was transferred to a hospital in the Zone of the Interior. There examination disclosed a distended abdomen with a slightly tender, hard spleen extending two cm. below and one cm. to the right of the umbilicus. Laboratory studies showed five grams of hemoglobin, and 1400 white cells with 47 per cent polymorphonuclears. A sternal biopsy showed a markedly hyperplastic marrow and many inclusion bodies suggestive of *L. donovani*. The total protein was 11.8 grams with a globulin of 7.5 grams and an albumin of 3.5 grams. The platelets were 200,000, the reticulocytes on several determinations were between seven and ten per cent, the icteric index was 12 and the bilirubin 1.3 mg. Frequent small transfusions were given and the patient was transferred to Walter Reed General Hospital for further therapy. There another splenic puncture was done and *Leishmania donovani* were demonstrated by direct smear, by culture on NNN media, and by inoculation into hamsters.

During the next 16 months, from 13 August 1944 to 11 December 1945, various types of therapy with antimony and diamidino compounds were tried with relatively little effect, as can be seen by referring to figure 1. The red cell count improved spontaneously only once; and the remainder of the time it was only by dint of 85 transfusions of 500 cc. each of whole blood that it was kept within the one to three million range. The white cell count ranged from 1000 to 4000 and the spleen remained below the level of the umbilicus, although some fluctua-

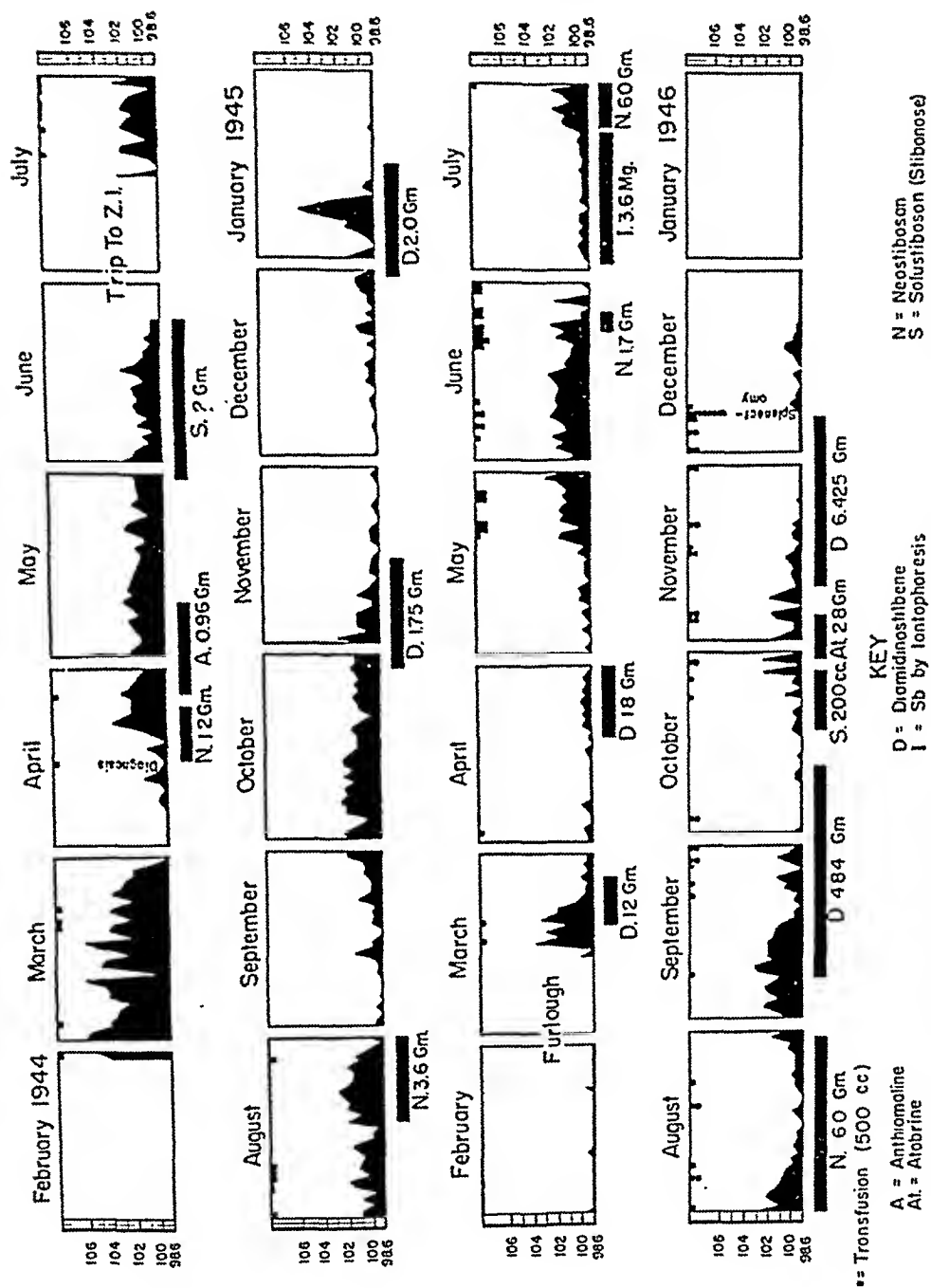


FIG. 1. TREATMENT AND ITS EFFECT ON THE HIGHEST DAILY TEMPERATURE VISCERAL LEISHMANIASIS

tion in the size of this organ, presumably resulting from treatment, was noted. The fever and the patient's general condition were temporarily improved from time to time, probably as a result of therapy, but relapses always followed.

In May 1944, the patient developed a stinging sensation of the left side of the face with slight diminution of perception of light touch, and trigeminal neuropathy (5) as a consequence of diamidino stilbene therapy was diagnosed. Further treatment with this drug was abandoned. During the latter part of May and June the neuropathy cleared but the patient's condition grew gradually worse. Several courses of neostibosan were given with no lasting benefit.

On 10 September 1945 the patient first came under the care of one of the authors. Physical examination at this time showed a hard, slightly tender spleen that descended into the pelvis on the left and the tip of which was approximately two cm. to the right and three cm. below the umbilicus. A rub could be heard over the spleen midway between the costal margin and the umbilicus. The patient's condition deteriorated rapidly and it was therefore decided to risk a long course of 4:4' diamidino stilbene, despite the fact that the patient had had a left sided trigeminal neuropathy four months before. Daily injections of 4:4' diamidino stilbene were continued for 34 days for a total of 4.84 grams of the drug. On the 25th day of therapy the temperature dropped to normal and the patient felt considerably improved. The spleen decreased two to three cm. the right border regressing to the midline at the umbilicus (see figure 2). The improvement, however, was short-lived, and the fever returned. Despite a course of stibanose, 20 cc. daily (each cc. representing 20 mg. of metallic antimony) for ten days, the spleen again increased in size, and the temperature rose to 102 and above with occasional chills. Although repeated blood cultures and malaria smears were negative, a clinical trial of quinacrine was given. This had no effect on the fever. Splenic punctures done on 20 October, 24 October, 7 November had all been negative on smear and culture, but the fluid obtained even from deep inside the spleen had not been satisfactory (at splenectomy a large 13 x 6 cm infarcted area was found on the anterior surface of the spleen which explains the occasional sharp, sliding pain over this area, the rub, and the lack of positive results on aspiration). Sternal puncture was done also with negative results. Despite all these negative results from diagnostic procedures another and more strenuous course of diamidino stilbene was begun, since no support for any other diagnosis was given by 15 negative blood cultures and the negative agglutinations.

On 13 November this drug was begun in a dosage of 200 mg. each day for seven days, after which the dosage was increased to 300 mg. each day until a total of 6.425 grams of 4:4' diamidino stilbene had been given over a period of 24 days. The temperature became normal on the 12th day of therapy and there was some improvement in the general condition of the patient. There was no reason to be sure, however, that this had effected a permanent cure; the transfusion demand continued (it had averaged 500 cc. of blood every three days for the past seven months), and the tremendously enlarged and somewhat painful spleen was making eating difficult.

On December 11, 1945 the spleen weighing 3050 grams was removed without undue loss of blood. Although the patient's course was stormy for the first 24 hours, rapid recovery followed. The hematologic changes were striking (see figure 3). The leukopenia disappeared immediately with the white cell count rising to 22,900 the evening after splenectomy. The last transfusion was given on the first postoperative day, but the blood rose to a level of 4.0 million red cells

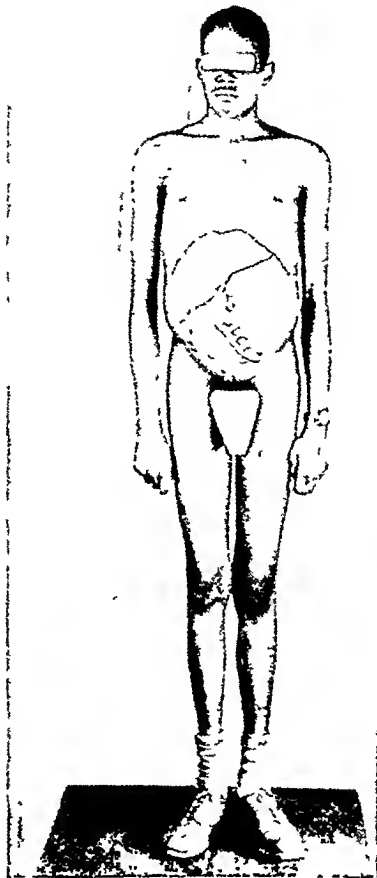


FIG. 2. DECREASE IN SPLENOMEGALY FOLLOWING COURSE OF DIAMIDINO STILBENE THERAPY IN SEPT. 1945

and 80 per cent hemoglobin and remained thus for the next six weeks after which it gradually rose to normal values.

PATHOLOGICAL REPORT

Gross: The spleen measured approximately 30 x 50 x 10-12 cm. and was irregularly oval in shape. The surface was dark purplish red. There was a grayish yellow, firm, roughly oval, 13 x 16 cm. infarcted area and four 2 x 6 cm. smaller infarcts were scattered over the surface. On section, the surface was rather firm, dark brownish red and nodular markings were obliterated. Sections

through the infarct showed a sharp line of demarcation from the surrounding splenic parenchyma.

Microscopic: There was considerable destruction of the splenic architecture by a diffuse reticulo-endothelial proliferation. There were areas of fibrosis and considerable phagocytosis of blood pigment. The sections through one of the infarcted areas showed partial replacement by fibrous tissue. Examination of

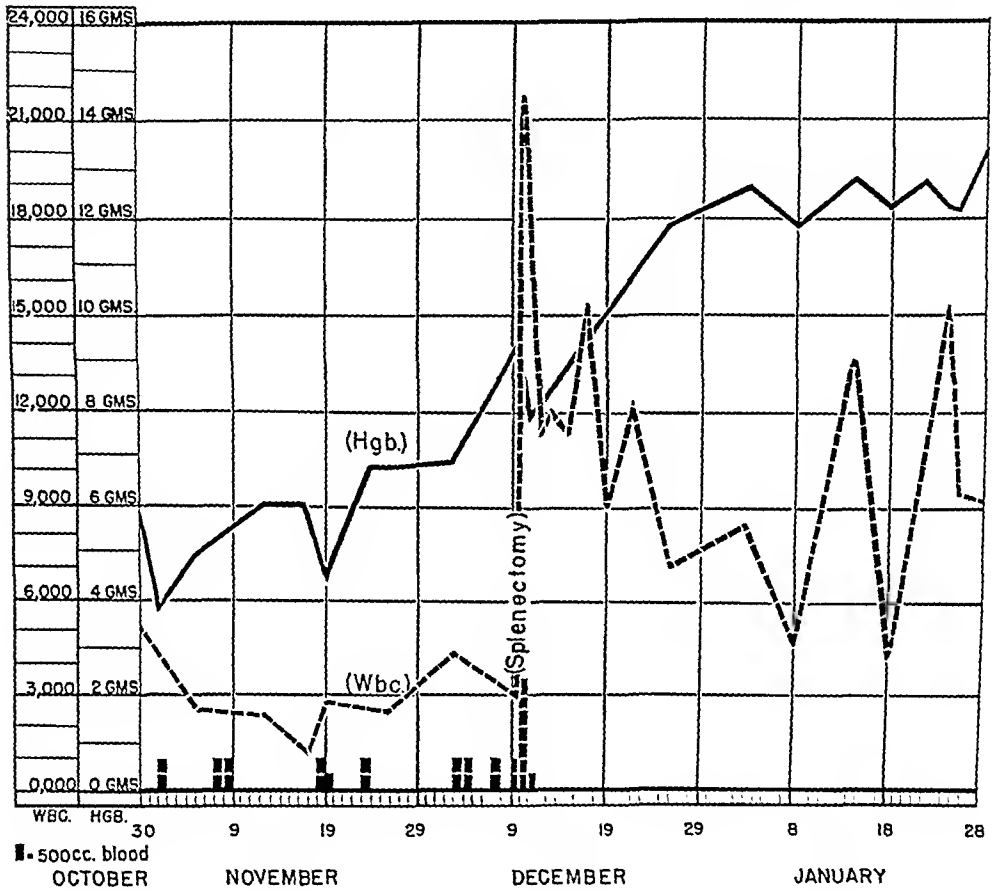


FIG. 3. THE EFFECT OF SPLENECTOMY ON THE WHITE BLOOD CELL COUNT AND HEMOGLOBIN

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one of the smears stained by the Giemsa method revealed frequent *Leishmania donovani* within the large endothelial cells. These bodies were also found in the permanent sections but were much less frequently or clearly demonstrated.

Diagnosis: Splenomegaly secondary to kala azar.

Six young hamsters inoculated with a heavy suspension of splenic tissue showed definite evidence of infection with *L. donovani* when examined 12 weeks later and leptomonads were obtained in cultures on NNN media from the spleens of these hamsters. Since the immediate post-operative period when he had a mild

upper respiratory infection, the patient has been afebrile, has gained weight rapidly (35 pounds in ten weeks) and has needed no further therapy. The blood has remained within normal limits. The proteins returned to normal at the seventh week and the formol gel became negative at the twelfth week after splenectomy. It has now been 16 months since splenectomy and despite the fact that he has had no further medical treatment since that time, the patient is now in good health.

DISCUSSION

In the handling of this patient many different therapeutic approaches, mainly unsuccessful, were tried, (see figure 1). It is felt that a discussion of the reasons for these attempts and failures is in order. The first course of neostibosan was entirely too short. Anthiomaline is recognized to be not particularly effective in combatting leishmanial infections. Solustibosan is known to be a most effective agent in this disease, but unfortunately no record is available of the dosage employed in this case. No reason can be given for its failure in this case unless the dosage was inadequate. The second course of neostibosan, 0.3 grams intravenously for 13 days, was in dosage which is usually adequate in the treatment of kala azar in India (3), but perhaps because of the previous antimony therapy, this too was inadequate. Whether antimony-fast strains of *Leishmania* can be developed through frequent inadequate dosage (6) is unproven, and Napier (7) feels this does not occur.

The total dosage of diamidino stilbene used in the first course was approximately the same as that used by Napier, Sen Gupta, and Sen in India (8) if allowance is made for the fact that we were using the di-isethionate salt which is 50 per cent heavier than the di-hydrochloride. In their series of 101 cases, 94 per cent permanent cures were obtained. Other investigators (9), (10), (11), however, have used much larger dosages particularly in cases from the Mediterranean area and the Sudan. The second course of the drug, 100 mg. for five days, then 150 mg. daily for ten days, was in more adequate dosage and a permanent cure was almost effected. The next two courses of 1.2 grams and 1.8 grams were totally inadequate. The first was discontinued because all available veins had been thrombosed by the drug, the second because of the appearance of a trigeminal neuropathy. This neuropathy has been reported as occurring two and one-half to five months after therapy with diamidino stilbene and is thought to be due to a toxic effect of the ethylene linkage on the fifth nerve nucleus in the pons (5). The short course of neostibosan, 0.5 grams daily, might have been in adequate dosage had it been continued, but the onset of generalized convulsions caused its discontinuance. The last course of neostibosan, 0.3 grams every second day for twenty injections, was certainly large in its total dosage, but perhaps it might have been more active therapeutically if the dosage had been given every day. That in this dosage the drug was ineffective is attested by the fact that even before the end of the 40-day course of therapy, the fever reappeared. There was no striking improvement in either splenic enlargement or the hematologic picture. The next therapeutic attempt, diamidino

stilbene therapy in a dosage of 150 mg. per day for 34 days, was larger than that recommended by Napier *et al.* (8) but was in line with the type of treatment used in Palestine by Susskind and Roth (9). The drug was discontinued after 34 injections because late toxic manifestations were feared in view of the previous history of trigeminal neuropathy. It appears possible in retrospect that perseverance in the therapy at this time might have effected a cure as some definite decrease in the size of the spleen and a disappearance of the fever were noted, (figure 2). After a week's break in therapy, stibanose, 20 cc. daily for ten days, was given, but the patient relapsed; the fever reappeared and the splenomegaly increased. Although this dosage is considerably larger than that used by many in curing kala azar (2), (12), (13), (14), (15), and should have been adequate in this case at least in causing a remission, no beneficial effect whatever was noted.

The final course of diamidino stilbene was given in massive dosages. The total dosage of drug given the patient during this course was 6.425 grams and the total over a period of 13 months was 18.015 grams. No ill effects whatever were noted from this dosage, (5 mg. per kg. of body weight) which is apparently the largest reported in the literature. Somers (10), Susskind and Roth (9), and Kirk and Sati (11) have used total doses as high as from 4.88 to 5.5 grams, but the daily doses were much smaller. That this high dosage was more effective is evidenced by the fact that the temperature dropped to normal on the twelfth day of therapy and remained normal until the fever of the post operative period. Despite the fact that the fever had disappeared, the spleen continued large and somewhat tender, and the need for transfusions to keep the blood at a level of 40 per cent hemoglobin remained unchanged.

The question at this point was whether or not to remove this patient's spleen. There were four aspects to the problem as follows: leishmaniasis, anemia, leukopenia, and the general discomfort of this tremendous splenomegaly. The recent fever, the increase in the size of the spleen on cessation of treatment, the leukopenia, the reversal of the albumin-globulin ratio, the strongly positive formol gel test, and the apparent temporary response to diamidino stilbene therapy all pointed to continued leishmanial infection despite three negative splenic punctures and one negative sternal puncture in the preceeding two months. If this patient had continuing leishmanial infection despite his more than adequate treatment, it might well be that the organisms lying deep in the enormous spleen were not reached by concentrations of drug adequate to destroy them completely. The correctness of this assumption seems to have been proved by the later splenic sections and bio-assays, and the post operative course.

The anemia was severe and 85 transfusions of 500 cc. each of whole blood had been needed in the preceeding months to maintain the blood at a level of 1.5 to 3.0 million red cells. The majority of the early transfusions were of type "O" blood but during the last two months all were type "A". These crossmatched perfectly. The type "O" blood matched perfectly on the major side. The patient's blood group was "A" and he was Rh positive. His serum showed no A₂ agglutinins. Even under such intensive transfusion therapy the red cell count rose only to 3.0 million and the highest hemoglobin recorded was 8.1 grams. In one week the patient had been given 4000 cc. of compatible type A blood all less than 72

hours old, but three days later the hematocrit was only 24 per cent, the red cells only 2 million, and the hemoglobin only 6.7 grams. The reticulocytes varied from 10 to 25 per cent during this time. There was a very slight increase in the osmotic fragility, hemolysis of the patient's cells beginning at 0.48 per cent saline, the control cells at 0.42 per cent saline. No sickling trait was found on many preparations. The icteric index varied from five to 12 with one isolated report of 25 in July 1945. All these data pointed to an anemia of the hemolytic type for which the following mechanism might be postulated. The leishmaniasis in causing an enlarged spleen and a great increase in the reticuloendothelial tissue provided three factors which might have contributed to the red cell destruction. The first would be the opportunity for the pooling of the blood in the enlarged spleen. This would in turn lead to increased hemolysis as postulated by Ham and Castle (16). The hyperglobulinemia, presumably the result of excessive hyperplasia of the reticulo-endothelial system (17) would cause pseudo agglutination and rouleau formation, (18). This in turn would contribute to increased erythrostasis in the spleen. The great increase of reticulo-endothelial tissue might in itself lead to increased destruction of the blood. Various investigators have felt that an increase of the reticulo-endothelial tissue in the spleen caused the leukopenia and anemia found in Banti's syndrome (19), (20), in tuberculosis of the spleen (21), and in lymphogranulomatosis (20). Most of these postulated a splenic influence on the bone marrow as the cause. Singer *et al.* (22) postulated that in some "hypersplenic" conditions the spleen has direct effect on the red blood cells which traverse the sinusoids and indirect effects on the hemopoietic cells of the bone marrow. Wiseman and Doan (24) have reported three cases showing profound granulocytopenia cured by splenectomy.

Since medical treatment cures from 90 to 98 per cent of the cases with far less risk, splenectomy as a therapy for acute kala azar is considered definitely contraindicated (24), (25), (26). Martin and Chorine (27), however, reported a case which did not respond to a course of 0.4 grams of neostibosan every second day for a total of 4.6 grams, but which after splenectomy responded rapidly to an unstated amount of the same drug. More recently Sweeney *et al.* (28) reported a case of kala azar simulating splenic anemia in which the diagnosis was not made until splenectomy and which recovered afterwards on a total dosage of 4.7 grams of neostibosan.

In both these cases there was a sudden increase in leukocytes (from 3200 to 5400 in the first and from 1800 to 12,000 in the second) immediately after splenectomy. These figures added to our case with an increase of from 2900 before splenectomy to a level of 22,900 immediately post operatively (see figure 3), followed by a gradual return to normal levels, would tend to show that the leukopenia in leishmaniasis is a function of the spleen rather than a crowding out of the bone marrow cells by the reticulo-endothelial cells and parasites. This effect of splenectomy in various conditions such as Banti's syndrome, tuberculosis of the spleen, and lymphogranulomatosis, conditions which have as a common denominator only a hyperplasia of the reticulo-endothelial tissue in the spleen, has been noted by many authors (19), (20), (21), (22), (23). There has been no satisfactory explanation as to the leukopenia in kala azar up to the present time

other than the possible crowding out of the bone marrow cells by a great proliferation of the cells of the reticulo-endothelial tissue and the parasites (29). The senior author (1) has now seen three patients (cases 1, 2, and 5) where the leukopenia was down to 1000 white cells but where adequate sternal aspirations of bone marrow were unable to demonstrate any parasites. This would seem to be against any crowding out or any local toxic action from parasites or parasite-laden cells in the bone marrow.

The relief of the anemia, whatever the explanation, was almost immediate. One month after operation the hematocrit was 44 per cent. A marked improvement in general comfort, appetite, and nutrition was noted after splenectomy.

It is well known that *Leishmania* may be found in splenic aspirations from patients with kala azar as long as six to eight weeks after the conclusion of a successful course of chemotherapy. Since, however, specific antimony and diamidine therapy over the twenty months before operation failed to effect a permanent cure and since this patient has now been well for 18 months following splenectomy without further specific therapy since three days before operation, it seems reasonable to conclude that the splenectomy, in addition to relieving the anemia and the leukopenia, may also have been of value in eradicating a hitherto inaccessible focus of leishmanial infection.

SUMMARY

1. Three patients with leishmaniasis previously reported have now been observed for 23 to 29 months following treatment and so can be presumed cured.

2. A fourth case of leishmaniasis has been discussed which was refractory to all usual dosages of neostibosan, stibanose, and diamidino stilbene. The anemia was so severe as to require the transfusion of 51 liters of whole blood during a period of 22 months. The leukopenia was severe and constant. The patient finally recovered following treatment by a combination of very massive dosage of diamidino stilbene and splenectomy. It seems reasonable to assume that the removal by splenectomy of a hitherto inaccessible focus of infection in the tremendously enlarged and infarcted spleen was instrumental in curing the patient.

3. The anemia and leukopenia disappeared following splenectomy.

4. This last fact leads the authors to postulate that the leukopenia in kala azar may be caused by the great proliferation of the reticulo-endothelial tissue in the spleen.

5. This report is not to be construed as suggesting the advisability of splenectomy in the usual case of visceral leishmaniasis.

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THE SIGNIFICANCE OF *ENDAMEBA HISTOLYTICA* IN STOOLS OF INDIVIDUALS WITH ACUTE DIARRHEA OF MODERATE SEVERITY*

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It has been pointed out by Craig (1) that the vast majority of cases of amebiasis do not show dysentery but manifest much milder symptoms usually attributed to some other factor and therefore not recognized as the result of infection with *E. histolytica*. Furthermore, Bartlett (2) has stated that when symptoms of dysentery do occur, there is already a condition of marked ulceration in the intestine. However, Craig (3) has also observed that in carriers with symptoms which he designates as Class II in severity, there is commonly observed a "very slight and evanescent diarrhea." This diarrhea may be painless or accompanied by colicky pains. It is free from gross blood or mucus and may persist for an hour or perhaps a day. It is never severe and the number of bowel movements does not exceed three or four a day, while the duration of the attack is rarely more than a day or two.

In the course of investigating possible etiological agents causing diarrhea among American troops stationed in and around Calcutta, India, it was noted that a relatively large number of these individuals showed *E. histolytica* in their stools. The number of cases was not of epidemic proportions and it was even unusual for large numbers of individuals from one organization to be affected at the same time. The cases studied showed only mild or moderately severe symptoms consisting of a sudden onset of diarrhea manifested by two to twenty liquid or semi-liquid stools per day; in most cases it varied between five and ten. The presence of gross blood or mucus was rare. There was generally some malaise; nausea was occasionally present but vomiting was infrequent. Abdominal cramps varied from none or slight to severe. In a few of the more severe cases, fever from 100 to 102° developed. If no specific treatment was instituted, recovery usually occurred in from one to five days. It was thought by some that sulfonamide drugs appreciably shortened the course of the disease. The symptoms were on the average slightly more severe than those designated as Class II in Craig's classification (3), but they were generally single episodes and not sufficiently severe to be designated as Class III. In the latter there are generally repeated attacks of diarrhea of considerable duration and severity.

Approximately 20 per cent of these individuals showed *E. histolytica* in their stools, an incidence as great as that of any other possible etiological agent isolated. Because a large percentage of American troops in the India-Burma theater

* The technical portions of these investigations were carried out by enlisted personnel of the 29th Medical Laboratory, Headquarters at Calcutta, India, Lt. Col. Perry T. Hough, M.C. Commanding.

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harboring this parasite showed no symptoms of diarrhea, and since, as a general rule, individuals with diarrhea apparently due to this organism alone had a more prolonged course which could be designated as Class III, there arose the question as to whether or not the ameba in these cases was to be considered the sole cause of the immediate diarrheal disease or if other factors might have an important role.

The problem was further emphasized when it was noted that in a similar study of outbreaks of diarrhea in American troops in the New Delhi area, the incidence of infection with *E. histolytica* was considerably lower but the incidence of other possible etiological agents was approximately the same as that obtained in the Calcutta study.

The data obtained from both of the above investigations are presented here because they are relevant to an analysis of the role of *E. histolytica* in the etiology of single episodes of acute diarrhea of moderate severity. They are also of importance in an analysis of possible associated factors such as bacterial infections in the pathogenesis of amebiasis.

MATERIAL AND METHOD

Stool examinations for enteric pathogenic bacteria and intestinal parasites were carried out in 219 individuals with diarrheal disease of the type described above who were stationed in the Calcutta area. In addition, 220 soldiers with no history of diarrhea received the same type of examination; 100 of these were from a unit without sporadic outbreaks of diarrhea and the remaining 120 from units in which patients with diarrhea were studied. Another group consisting of 590 individuals from a number of other units were studied for parasites only, in order to further establish the incidence of *E. histolytica* among American troops in this area.

In 144 of the diarrhea cases and in the 220 controls, only one stool examination for both parasites and pathogenic bacteria was made. A single stool examination on two successive days was carried out in 35 persons and for three successive days in the remaining 39 individuals with diarrhea. In one case of diarrhea with *E. histolytica*, no culture was obtained. In the New Delhi group, 263 soldiers with diarrhea and 105 control subjects received a single parasitological examination; all of the controls and only 235 of the diarrhea cases received a single bacteriological examination. Successive cultures were not obtained in New Delhi.

Material for stool cultures was obtained from the rectal wall by the Hardy swab technique (4) and immediately plated on E.M.B. and S.S. agar; the swab was then placed in Selenite F. broth. A second S.S. agar plate was inoculated with material from the passed stool and some of the latter was also placed in the Selenite F. broth containing the rectal swab. Material from the Selenite tubes was usually streaked on an additional S.S. agar plate after incubation for 18 hours. Kligler's iron agar was used to group the non-lactose-fermenting organisms in appropriate cases. The urease test as described by Anderson (5) was used to eliminate *Proteus* sp. and the Paracolon sugar medium described by

Felsenfeld and Young (6) was employed to identify the Paracolon group of organisms. The latter were then classified as either *Aerobacter*, Intermediate or *Escherichia* by the IMViC formula as described by Parr (7). Fermentation sugars and specific agglutinating sera were used to identify *Shigella* and *Salmonella* sp. Examinations for *Vibrio cholera* were routinely carried out in the first 100 diarrhea cases in each group but no positive cultures were obtained.

Parasitological examinations were carried out in freshly passed specimens. A saline suspension and an iodine-stained preparation were immediately studied. In addition, an iron-alum Hematoxylin stain of stool films from each case prepared according to the rapid staining method described by Paschal (8) was studied.

TABLE 1

A Comparison of the percentage incidence of various amebae in diarrheal and non-diarrheal cases

SPECIES OF AMEBAE	CALCUTTA		NEW DELHI	
	Diarrheal	Non-diarrheal	Diarrheal	Non-Diarrheal
<i>E. histolytica</i>	31.5(24.2)*	20.5	4.7	1.9
<i>E. coli</i>	8.2	9.8	6.4	7.6
<i>E. nana</i>	5.5	16.4	6.0	6.7
<i>I. butschlii</i>	2.3	3.8	0.9	2.9
<i>D. fragilis</i>		0.1	0.4	1.0
Total cases.....	219	810	267	105

* Incidence of *E. histolytica* when prolonged and/or repeated diarrheas are eliminated.

RESULTS

Incidence of Amebiasis

The incidence of amebiasis in 810 American soldiers as determined in surveys of a number of units in the Calcutta area was 20.0 per cent; the incidence in the 219 individuals with diarrhea was 31.5 per cent. However, 21 of the 69 individuals with amebiasis in the latter group either gave a history of diarrhea in excess of five days or of repeated attacks, and should therefore not be considered in the present study. Elimination of these cases results in an incidence in the diarrhea group of 24.2 per cent, a figure which is not significantly greater than that found in cases of asymptomatic amebiasis.

The incidence of *Endameba histolytica* among American soldiers in the New Delhi area was so low in both diarrhea and non-diarrhea groups that no attempt is made to draw conclusions from these figures. However, in table 1, we have listed the incidence of the various species of amebae in diarrhea and non-diarrhea cases in both groups in order to indicate the completeness of the examinations. A number of other intestinal protozoa and helminth ova and larvae were encountered, but these have no bearing on the present investigations.

In table 2, a correlation is attempted between length of service in the theatre

and the incidence of the amebiasis. Except for a slight increase in incidence in persons stationed in the I. B. theatre for twenty months or more, there is no significant correlation between length of service and incidence of amebiasis. The results indicate that in most instances there was probably early unrecognized infection with *Endameba histolytica*.

TABLE 2

*Relationship of incidence of amebiasis to length of service in I-B theatre
(554 survey cases by Calcutta group)*

LENGTH OF SERVICE	NO. INDIVIDUALS	NO. OF CASES AMEBIASIS	PER CENT INCIDENCE
1-7 months	190	38	20.0
8-13 months	246	43	17.5
14-19 months	94	18	19.1
20 months and over	24	7	29.1

TABLE 3

Bacteria isolated from the stools of acute diarrhea and non-diarrhea cases*

ORGANISM	CALCUTTA				NEW DELHI			
	Diarrhea		Non-Diarrhea		Diarrhea		Non-Diarrhea	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
<i>Shigella</i> , sp.....	46	27.5	9	4.1	48	20.2	10	9.5
<i>Salmonella</i> , sp.....	1	0.5	0		8	3.4	3	2.9
<i>Eberthella</i> (not <i>typhosa</i>) sp.....	2	1.1	0		15	6.3	2	1.9
Paracolon.....	79	47.3	119	54.1	70	29.1	38	36.2
None of above.....	55	32.9	92	41.8	120	50.6	58	55.2
Total cases†.....	167		220		237		105	

* Cases of diarrhea of longer than 5 days duration or with a history of repeated attacks are not included in this table.

† The difference between the figures listed in "Total Cases" and the sum of the column above them is due to cases with combinations of organisms listed.

Pathogenic Bacteria in Diarrhea Cases

In table 3, we have compared the bacteriological findings in cases of diarrhea of short duration and in controls in the Calcutta and New Delhi areas. These data are presented to show that despite the divergent incidences of amebiasis in the two zones, the bacteriological findings are similar in a number of important respects: (1) The incidence of infection with *Shigella* sp. is only slightly higher in the New Delhi area than in Calcutta; (2) *Salmonella* and *Eberthella* occur infrequently in both groups although the incidence is slightly higher in New Delhi; (3) Paracolon infections occur more frequently in the Calcutta group, but there is also a strikingly high incidence of this organism in controls in both groups. The latter is higher in the Calcutta group. Additional data concerning Paracolon organisms is presented below.

Enteric Pathogenic Bacteria Associated with Endameba histolytica in Diarrhea Cases

In table 4 we have compared the bacteriological data in the diarrhea cases with amebiasis with those in which *Endameba histolytica* are not found. The data in prolonged cases is presented for the sake of completeness but there are too few cases to draw any significant conclusion. In the cases of the diarrhea of short duration, there is a striking similarity in the incidence of various bacteria in amebiasis and non-amebiasis cases. *Shigella* occurs only slightly more and Paracolon only slightly less frequently in diarrheas with amebiasis than in diarrheas in which this protozoan was not found.

The high incidence of Paracolon in diarrhea and control cases deserves further analysis. These organisms have been divided by means of the IMViC formula and the distribution compared with that in a group of Indian food handlers with-

TABLE 4

A comparison of possible pathogenic organisms isolated from diarrhea cases, with and without amebiasis

ORGANISM	AMEBIASIS CASES				NON-AMEBIASIS CASES			
	Diarrhea of Short Duration		Diarrhea of Long Duration		Diarrhea of Short Duration		Diarrhea	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
<i>Shigella</i>	14	30.0	6	28.6	32	26.7	5	16.7
<i>Salmonella</i>	1	2.1	0		0		2	6.7
<i>Eberthella</i>	0		0		2	1.7	0	
Paracolon	20	42.6	7	33.3	59	49.2	15	50.0
None of above	16	34.0	9	42.9	39	32.5	13	43.3
Total cases*	47		21		120		30	

* The differences between the figures listed in "Total Cases" and the sum of the column above them are due to cases with combinations of organisms listed.

out diarrhea. It should be noted that the controls were individuals who had been stationed in India for only three or four months.

The controls in both areas show a preponderance of the *Escherichia* group and there is a marked increase in the incidence of the Intermediate I variety in diarrhea cases. Intermediate I also predominates in Indian food handlers.

Relation to Infecting Organisms to Exudate in Stool

All stools in these diarrheas were either fluid or semi-formed and many flecks of mucus or yellow patches of pus or gross blood were noted. It can be noted in table 5 that in stools in which there was no gross evidence of exudate, erythrocytes and polymorphonuclear leukocytes could be found frequently on microscopic examination. In both groups under investigation, a high percentage of *Shigella* and *Salmonella* infections showed both red and white cells. It should be particularly noted that the stools in a high percentage of cases with amebiasis contained polymorphonuclear leukocytes although no enteric pathogenic bacteria

could be demonstrated in these individuals. In cases with Paracolon and in negative cases, there was approximately the same proportion of individuals showing red and white cells although the percentage was not as high as in cases with *Shigella*, *Salmonella* or *Endamrba histolytica*. In the Calcutta group no

TABLE 5

An analysis of paracolon distribution on the basis of the IMViC reaction

PARACOLON TYPE	CALCUTTA						NEW DELHI			
	Controls		Diarrhea Cases		Indian Food Handlers		Controls		Early Diarrhea cases	
	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent	No.	Per cent
<i>Acrobacter</i>	28	19.4	14	11.3	13	20.9	8	21.1	20	29.4
Intermediate I*.....	14	9.7	55	44.3	26	41.9	5	13.2	19	28.0
Intermediate II†.....	29	20.1	15	12.1	8	12.9	6	15.8	4	5.8
<i>Escherichia</i>	73	50.7	40	32.3	15	24.2	19	50.0	25	36.8

* Intermediate I produce Indol.

† Intermediate II do not produce Indol.

TABLE 6

Relationship of infecting organism to exudate in stools

ORGANISM	NO. CASES	RBC		WBC		MONONUCLEAR MACROPHAGES	
		No.	Per cent	No.	Per cent	No.	Per cent
1. Calcutta Group							
<i>Shigella</i>	21	17	81	19	95		
<i>Salmonella</i>	2	2	100	2	100		
Paracolon.....	57	36	63	32	56		
<i>E. histolytica</i>	20	19	95	18	90		
Negative.....	47	29	62	30	64		
2. Delhi Group							
<i>Shigella</i>	29	17	59	26	90	15	52
<i>Salmonella</i>	4	2	50	4	100		
<i>Eberthella</i>	8	5	63	5	63	3	38
Paracolon.....	32	8	25	16	50	4	13
<i>E. histolytica</i>	5	4	80	3	60		
Negative.....	101	28	28	60	59		

data was tabulated as the frequency of the macrophages but it was noted that they were not present in amebiasis cases with diarrhea and negative stool cultures for bacteria. They were frequently present in cases with *Shigella* and occurred with moderate frequency in cases with Paracolon having symptoms of diarrhea. The more specific data tabulated by the Delhi group is also shown in table 6.

The frequent presence of polymorphonuclear leukocytes in most of the cases of diarrhea with amebiasis and negative stools for enteric pathogens and in the

negative cases, indicates the presence of a non-protozoan agent which we were unable to isolate. Furthermore, it indicates, in addition to the statistical data, the presence of a non-amebic agent producing diarrhea. The polymorphonuclear leukocytes in these cases were well preserved and were present in large numbers.

Relationship of Previous Diarrhea to Infection with E. histolytica

An attempt was made to correlate the history of previous diarrhea in a large survey group with the incidence of amebiasis. The results are shown in table 7. A history of previous diarrhea lasting more than one day was given in slightly less than half of the persons surveyed and showed no correlation with the presence of *E. histolytica*. Thus, in over half of the individuals infected with this organism, there was no history of previous diarrhea nor any awareness of this disease. Furthermore, no significant differences were noted when a history of multiple bouts of diarrhea was obtained.

TABLE 7

Relationship of previous history of diarrhea to incidence of amebiasis (554 survey cases by Calcutta group)

	PER CENT OF	
	Amebiasis cases	Non-Amebiasis
History of previous diarrhea.....	40.5	48.4
History of multiple episodes of diarrhea.....	17.0	22.4

DISCUSSION

The data presented here give interesting information on the incidence of amebiasis in American troops stationed in the Calcutta area of the India-Burma theater and the relationship of *E. histolytica* to single episodes of diarrhea of moderate degree. The data show an incidence of 20 per cent in individuals without symptoms of diarrhea and of 31.5 per cent in individuals with diarrhea. However, if the chronic cases are eliminated from the latter group, the incidence is then 24.2 per cent. This finding is in agreement with that of Payne (9) who has also observed a 20 per cent incidence of amebiasis in British troops stationed in eastern India. The incidence in the present investigations is almost three times as great as the average of all surveys in the United States as compiled by Craig (10), although included in his group are a number of surveys with a higher incidence. The latter are generally in American Indians, in rural populations or in institutions. It is unlikely that one out of five American soldiers was already infected with *E. histolytica* before arrival in India.

The attempt at correlating length of service with incidence of amebiasis showed no significant difference between length of service and incidence except for a slight increase in the group of persons stationed in the India-Burma theatre for twenty months or more. This fact coupled with the lack of history of previous diarrhea in many individuals with amebiasis indicates that persons may

be infected with this organism for long periods of time without awareness of disease. It also indicates that in all likelihood many soldiers became infected with *Endemeba histolytica* shortly after arrival in the theater.

A rather striking similarity in the bacteriological data was found when diarrhea cases with amebiasis were compared with those without this parasite. Furthermore, in clinically similar cases studied in New Delhi, the bacteriological findings were similar to those obtained in Calcutta while the incidence of *E. histolytica* was considerably lower. All of this evidence points to the conclusion that the diarrheas studied in these investigations were due to causes other than amebiasis and that the discovery of the latter was in all probability a concomitant finding.

As to the possible etiology of the diarrheas other than *E. histolytica*, in about one-quarter of the cases *Shigella* or *Salmonella* sp. were isolated and may be accepted as the etiological agent; in slightly less than one-half of the diarrhea cases in Calcutta and in about one-third of those in Delhi, Paracolon organisms were isolated. A high percentage of this type of organism was also isolated from non-diarrhea cases. However, when these organisms are classified according to the IMViC formula, there is a distinct difference between diarrhea and non-diarrhea cases. There is a preponderance of Intermediate I in Indian food handlers and a marked increase in this type in Americans with diarrhea, while in the Calcutta area in American controls, *Escherichia* predominates. The distribution of the Paracolon types in controls is comparable to that observed by Stuart, Wheeler, Rustigan and Zimmerman (11) and also by Michael and Harris (12), the former investigating Americans in the United States and the latter Americans in the Pacific Theater of Operations. The evidence is suggestive that the Indian food handlers are carriers acting as a source of infection of the American troops. A number of workers referred to by the above investigators have presented suggestive evidence of the pathogenic nature of the Paracolon group but the evidence is not yet conclusive. The frequent presence of polymorphonuclear leukocytes and the occasional presence of macrophages also suggests the pathogenic nature of these organisms.

In the present study there was a large group of individuals with diarrhea, with and without *E. histolytica*, who showed large numbers of polymorphonuclear leukocytes in their stools but from whom it was not possible to obtain cultures of enteric pathogenic bacteria. Callendar (13) states that a presumptive diagnosis of bacillary dysentery can be made by finding polymorphonuclear leukocytes in wet, unstained stool preparations. . . . "A higher proportion of positive results than one may ever hope to obtain by bacteriological methods." This statement serves to emphasize the fact that it is common experience even in epidemic outbreaks of diarrhea to find a large number of cases with cellular exudate of the type commonly seen in bacillary dysentery in which cultures for the enteric pathogenic bacteria are negative. There exists the possibility of other etiological agents as, for example, filterable viruses which are not sought for in routine bacteriological surveys. The cellular exudate in negative cases indicates an etiological factor independent of the presence of *E. histolytica*.

There remains to be discussed the possible relationship of the present bacterio-

logical findings to the symptomology in amebiasis. The fact that many cases with *E. histolytica* are asymptomatic raised the question many years ago as to whether or not this organism was pathogenic. Experiments in animals have amply demonstrated the pathogenicity of *E. histolytica* and the non-pathogenicity of other species of amebae. In 1894, Druse and Pasquale (14) produced typical amebic dysentery in a cat by rectal injection of bacteriologically sterile pus from a liver abscess containing amebae, thus demonstrating beyond doubt that *E. histolytica* and not bacteria was the cause of the disease. Furthermore in 1927, Craig (15) demonstrated the presence of hemolytic and cytolytic substances in extracts of cultures of *E. histolytica* which were not of bacterial origin but evidently secreted by the amebae.

Craig (16) and other investigators have maintained that many so-called healthy individuals have low-grade symptoms referable to the gastro-intestinal tract and other organs. In Craig's experience, 65 per cent of the individuals passing cysts of *E. histolytica* presented some symptoms that may have been caused by this parasite while the remaining 35 per cent were symptomless. Other observers have reported even higher percentages of symptomless carriers but some of these reports may have been based on cursory data as regards the presence of mild or indefinite symptoms. A rather detailed study in naval personnel of this aspect of the subject has been presented by Sapero (17) but even this investigator found a residuum of 46.6 per cent in which minimal symptoms referable to the gastro-intestinal tract and other organs are lacking. Furthermore, in the classical experiments by Walker and Sellards (18) in 1913 who fed *E. histolytica* cysts to 20 human volunteers in the Phillipines, 18 became parasitized but only 4 (22.2 per cent) developed clinical amebic dysentery.

Craig (16) maintains that pathological lesions are produced in every individual harboring this parasite, however minute these lesions may be. He further believes that whether symptoms of such invasion are present or not, depends on the resistance of the individual to the infection and it is probable that in many cases the minute lesions heal rapidly and the organism perishes. Still other investigators maintain that there is a variable virulence depending on the infecting strain (Meleney and Frye) (19) and Brumpt (20) believes that there are non-pathogenic varieties such as *E. dispar*, morphologically resembling *E. histolytica*. On the other hand Craig (3) states that if resistance is lowered for any reason, many of the lesions do not heal and symptoms of greater or lesser severity ensue. In the diarrheas presented in the present series, there may be such a factor of bacterial origin which lowers the resistance and allows the amebae to gain a firmer hold. Unfortunately there are no follow-up data which might be used to show an increased severity of symptoms due to *E. histolytica* in these cases.

However, in addition to lowering the general resistance of the patient with amebiasis, bacterial infections in the intestinal tract may play a more specific role in the pathogenesis of amebiasis. Nauss and Rappaport (21) have pointed out that it has not been satisfactorily proved that *E. histolytica* alone, utilizing the cytotoxin, is effective in accomplishing the initial mucosal penetration. Vari-

ous other investigators have shown that accessory factors may play a very important role in facilitating the breakthrough of the mucosal barrier by amebae. The evidence of the importance of bacteria as such an accessory factor has been reviewed by Nauss and Rappaport who have also presented additional evidence that bacteria in association with trauma of a toxic nature facilitates the penetration of the mucosa by *E. histolytica*. The bacteria isolated in the present investigations may be an important factor in determining the ability of amebae to penetrate the mucosa and the extensiveness of the intestinal ulcers.

SUMMARY

Data are presented to show that there was about a 20 per cent incidence of amebiasis among American soldiers stationed in the Calcutta area of the India-Burma theater. This incidence was existent in groups present in the theater for a relatively few months and did not increase significantly in groups present for longer periods. The incidence of amebiasis in American troops in the New Delhi area was only 5 per cent. Bacteriological studies for pathogenic enteric bacteria from the stools of patients with diarrhea of moderate severity showed a striking similarity whether or not *E. histolytic* was present. The bacteriological findings of the Calcutta and New Delhi groups were similar despite the striking difference in the incidence of amebiasis.

Evidence is presented to show that certain Paracolon types among individuals with diarrhea may be pathogenic, and cytological studies in stools of diarrhea cases further indicate a non-amebic etiology of the diarrheas studied.

From the data presented, it is concluded that the diarrheas of moderate severity studied in these investigations may be of a non-amebic origin and that the incidence of amebiasis (20 per cent) is probably a coincidental finding. It is further pointed out that the pathogenic bacteria and those of possible pathogenicity may play an important role in determining an individual's general resistance to an amebic infestation and it may also be an accessory factor in determining the penetrability of the amebae through the intestinal mucosa.

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A CASE OF SPRUE MAINTAINED ON FOLIC ACID

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Numerous observers have reported the treatment of macrocytic anemias with folic acid with the return of the blood to normal. Several (1-6) have found that the macrocytic anaemia of sprue was similarly improved. But so far, no one has reported the effect of folic acid in the maintenance of sprue over many months. For this reason, the fact that five milligrams of folic acid by mouth daily has been sufficient to maintain an old case of sprue in continued health for over one year, should be recorded.

CASE HISTORY

Diarrhoea commenced in 1924, twelve years after two periods of service in the Philippines. At first the diarrhoea was caused by acids like stewed tomatoes, and could be controlled by a rigid diet, but during a third tour in the Philippines in 1925-1928, the diarrhoea of a watery character became continuous, and following an episode of sore mouth which lasted only one week, the diagnosis of sprue was made. Some improvement followed the use of a low fat diet, and eliminating all acid fruits. But soon no diet could control the diarrhoea, and Tinct. Opii Camph and Bismuth subcarbonate were largely used with temporary relief. This condition lasted for years with some remissions, and alteration of the acute watery diarrhoea to the large, light colored foul stools of chronic sprue.

By 1935, the patient who normally weighed 140-145 pounds was reduced to 120 pounds, and felt definitely weak and very tired. At this time, patient entered a hospital and had a complete check-up, including proctoscopic examination, radiological gastro-intestinal series and chest, gastric examination, gall bladder series, and all ordinary laboratory and physical examinations. Briefly, the stomach contained a normal amount of hydrochloric acid, and all other findings were normal except 1. a large amount of fat and fatty acids in the stool; 2. A macrocytic anaemia with a count of 1,500,000 erythrocytes and a low leucocyte count with a normal distribution. 3. A habitual low blood pressure of 95-100 systolic and 45-60 diastolic.

At this time, the diagnosis of sprue being confirmed, patient was given 1 c.c. Liver Extract daily. (The Lederle crude product, 3 c.c. ampoules in box of three, each cubic centimeter containing 3.3 units.) Within one week stools became formed and a good reticulocyte count was obtained. However stools continued to be very large, very fatty and light colored. Patient remained in the hospital for three months receiving 1 c.c. of the same extract IM. daily. By this time blood had returned to normal, and all weight lost had been recovered.

At several times during this period liver extract and ventriculin by mouth were tried, but the response was poor, and diarrhoea would recur. Therefore Liver Extract IM. was continued throughout hospitalization.

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Leaving the hospital, patient continued to take the same Lederle Liver Extract I.M. gradually decreasing the dose. During the following years it was found that stools were generally formed when 1 c.c. liver extract was given bi-weekly, maintaining the diet high in protein, low fat, moderate carbohydrate and no acid fruits. Bananas were especially well tolerated. But any indiscretion in diet would bring on diarrhoea again and necessitate daily liver injections.

This condition continued until June 4, 1946 when synthetic folic acid was obtained from Lederle, and tried in a five milligram dose by mouth daily. It was found that folic acid (Folvite, Lederle) controlled the diarrhoea much better than liver extract. It also kept the blood normal, counts made in 1947 averaged haemoglobin 95% or 16.9 grams, erythrocytes 4,570,000, leucocytes 7,650 with a normal distribution. No abnormal cells. Since using folic acid, stools have continued to be well formed, and part of the time have been normal but at most times have continued to be abnormally large and fatty. Weight has continued at the normal variation of 140-145 pounds. The blood pressure has continued low, 100/60. In brief, this is an account of an old case of sprue maintained in good health for thirteen months by the simple ingestion of five milligrams of synthetic folic acid daily.

DISCUSSION

Sprue appears to be essentially a lack of intestinal absorption especially of fats and sugars. It has been suggested (7) that the loss of ability to absorb fatty acids, glycerol and glucose is due to a failure of phosphorylation, and to a loss of phosphorus as the result of failure of phospholipid formation.

The real question here is not the origin of sprue, but the mode of action of folic acid (Pteroyl-glutamic acid) which is still unknown. It may be related to the erythrocyte maturing factor stored in the liver. It is impossible that they are identical for the amount of liver extract that is effective in sprue does not contain an effective dose of folic acid. We have seen these chemical relationships several times. Synthetic vitamin K is more active than the more complex natural vitamin, and we know of at least three different vitamin D formulae with somewhat different actions.

It has been suggested that folic acid is concerned in the formation of thymine, but this seems highly improbable. Thymine has been found effective in pernicious anaemia, but only in relatively enormous doses compared with the great activity of folic acid.

How can either liver extract or folic acid control the diarrhoea and produce formed stools, while intestinal absorption is still in abeyance as indicated by the 'large fatty stools'? Several articles (8-11) indicate that in all macrocytic anaemias acetyl-choline is greatly increased and it is claimed that macrocytic anaemia can be produced in dogs by injections of acetyl-choline. When either liver extract, ventriculin or folic acid is administered, a very high concentration of cholinesterase is formed, and this esterase breaks up the acetyl-choline. According to Best and Taylor (12) acetyl-choline causes excitation of the parasympathetics, excitation of the muscles of the intestinal tract, dilatation of the arterioles

and a fall of blood pressure. Acetylcholine is one thousand times more active than choline. Acetylcholine is rapidly hydrolyzed by cholinesterase contained in blood and other bodily fluids.

According to this theory, in sprue and other macrocytic anaemias, acetylcholine is increased resulting in diarrhoea, and the esterase is decreased or missing entirely. Either liver extract or folic acid produces a strikingly high level of cholinesterase which is maintained for a time but gradually falls to normal limits as the blood returns to normal. Since abnormally high acetylcholine will produce diarrhoea and macrocytic anaemia, and either liver extract or folic acid destroys the acetylcholine by increased esterase, it is evident that the diarrhoea of sprue can be corrected by folic acid in spite of the fact that the intestinal condition that results in steatorrhoea is uncorrected and still present.

CONCLUSION

Folic acid in five milligram doses by mouth daily will maintain sprue in health permanently. One five milligram tablet daily may be considered the maintenance dose for even an old case of sprue.

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SEQUELAE OF JAPANESE B ENCEPHALITIS^{1, 2, 3}

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During the course of a recent investigation by the U. S. Naval Medical Research Unit No. 2 on the epidemiology of Japanese B encephalitis on Okinawa, the writers were afforded an opportunity to study a group of patients for clinical evidence of residual damage to the central nervous system resulting from infection with this neurotropic virus one year before (²). The findings are of interest because they provide data on the frequency, type, and severity of sequelae in a previously well-studied group, and make possible, to a certain extent, a comparison of the residual damage caused by the Japanese B encephalitis virus with that seen following other types of encephalitis.

An epidemic of Japanese B encephalitis which affected the civilian population of Okinawa and, to a limited extent, the American military personnel on the island (1) began abruptly in early July, 1945, and persisted until the middle of September. The first recognized cases were encountered on the tiny off shore islands of the Heanza Retto, but the majority occurred in rural areas of northern Okinawa proper, where numbers of civilians had been herded for safety and to prevent their interference with military operations in the south.

A large number of civilian cases were subjected to careful clinical and laboratory studies during the acute and early convalescent phases of the disease by several groups of investigators and in many instances the diagnosis was proved by serological procedures (2, 3, 4). These cases originally studied included 66 hospitalized at the U. S. Naval military government research center on Okinawa and an additional 36 cases on the Heanza Retto. The present study was made nearly a year later, in June and July of 1946, on 18 of the 53 surviving cases in the former group and 20 of the 24 survivors in the latter. At this time the con-

¹ This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth in this article are not to be considered as reflecting the policies of the Navy Department.

² Grateful acknowledgement is made to Comdr. F. A. Butler, MC, USN, Commanding Officer of U. S. Naval Medical Research Unit No. 2 at the time of this investigation; Capt. W. H. Harrell, MC, USN, then Senior Medical Officer, Naval Operating Base, Okinawa; and to the personnel attached to U. S. Naval Military Government, Ryukyus, particularly Lt. Comdr. J. J. Halbert, MC, USNR, then Senior Medical Officer of that activity. Valuable assistance was given by Lt. L. M. Miller, MC, USN, in the examination of cases from the Heanza Retto, where he first recognized the presence of the disease in 1945. The previous clinical data on cases occurring on Okinawa proper were available through the courteous cooperation of Comdr. Leon Lewis, MC, USNR, formerly attached to U. S. Naval Military Government, Ryukyus.

³ Publication cost defrayed by assistance of a grant from the Coolidge Foundation to the American Society of Tropical Medicine.

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fusion of relocating the whole population made it impossible to find a larger number. Evaluation of each individual patient was made after a general physical examination, a neurological examination, and a mental status examination. The last procedure, admittedly a difficult one, was made reasonably reliable through the efforts of an extremely able Okinawan interpreter.

CLINICAL DATA

During the acute phase the disease was characterized by fever, headache, altered state of consciousness (lethargy, stupor, delirium, or coma), nuchal and spinal rigidity with associated signs of meningeal irritation, altered cutaneous and tendon reflexes, focal motor weakness, and the occasional presence of pathological reflexes, dissociated ocular movements, athetosis, convulsions, tremors, or aphasia (2, 3).

TABLE 1
Analysis of findings in eleven patients with neurological sequelae

PATIENT	AGE	SEX	MOTOR FUNCTION	MENTAL STATUS
KS	4	M	Athetoid gesture, right arm	Normal
MM	5	M	Weakness, left arm and leg	Mental retardation
TN	5	M	Quadriplegia	Advanced deterioration
TH	7	F	Rt. hemiplegia, Partial paralysis cranial nn. 10, 11, 12	Aphasia
IK	9	F	Normal	Aphasia
THi	13	M	Incoordination, right hand	Normal
IS	14	M	Weakness, right arm and leg	Normal
IA	14	F	Weakness, left leg	Mental retardation
TK	15	F	Normal	Mild personality change
GT	15	F	Normal	Mental retardation
YC	21	F	Incoordination, both legs	Mental retardation

When seen ten to twelve months after onset of the disease, eleven of the thirty-eight patients examined had clinically detectable neurological sequelae. The types of sequelae observed are present in table 1. Ten of the eleven fell within the age group ranging from four to fifteen years, this group totalling 31. or 77% of the patients examined. The remaining nine patients, 23% of the total, ranged from seventeen to sixty-six years of age. One of these, 21 years of age, displayed slight mental retardation and incoordination of the lower extremities. Severely incapacitating sequelae were encountered in only three patients. Case histories of these patients and of two others presenting less evidence of residual damage are summarized below (5):

Case 1: The patient (TN) was a five-year old male Okinawan who had been admitted to the hospital on August 29, 1945, the fourteenth day of his illness, in a lethargic state and suffering from a severe headache, stiff neck, fever, weakness of extremities, and loss of speech. The cerebrospinal fluid was clear and contained 50 leucocytes per cu.mm. (95% mononuclear cells), 85.2 mgm. total protein per 100 cc., and 50 mgm. sugar per 100 cc. Complement fixation and

neutralization tests were reported as "positive" for Japanese B encephalitis. The patient became afebrile shortly after admission, but proceeded steadily into a vegetative state with paralysis and contractures of both legs.

When examined on June 20, 1946, ten months after onset, the child was still hospitalized as a helpless bed patient. There was marked pallor of the skin and mucous membranes, and slight pitting edema over the ankles. The extremities showed diminished strength and slight spasticity. Atrophy and contractures of both legs were present. The cranial nerves were intact, cutaneous and tendon reflexes were present and equal, plantar reflexes were normal, and there was no incontinence. Aphasia was present and the patient responded only to certain stimuli such as pain and bright light, but was able to chew and swallow. Attendants stated that there had been little improvement.

Case 2: This patient (TH) was a seven-year old female Okinawan who had been admitted to the hospital on July 10, 1945, six days after onset of her illness. Initial symptoms had been headache, fever, and increasing stupor. On the day prior to admission she had had several convulsions of a generalized nature and then had lapsed into coma. Examination at the time of admission revealed fever, tachycardia, nuchal rigidity, positive Kernig sign, unequal tendon reflexes and absent abdominal cutaneous reflexes. Plantar reflexes were normal. The cerebrospinal fluid was clear and contained only 6 mononuclear cells per cmm., with a negative Pandy test for protein. The patient regained consciousness after five days in the hospital, but showed a residual aphasia with considerable irritability. Hemiparesis of the right side became apparent as spontaneous movements improved. On October 22, a little over three and a half months after onset, spasticity and hemiparesis of the right side, aphasia, and excessive salivation were noted. A neutralization test was reported as positive for Japanese B encephalitis and a definite rise in titer of complement-fixing antibody was demonstrated.

Subsequent examination on July 7, 1946, one year after onset, revealed essentially the same condition to be present. The patient was able to walk with the typical gait of the hemiplegic individual and showed considerable ankylosis of the right ankle. There was constant drooling of saliva from the mouth. In addition it was noted that the pharyngeal reflex was suppressed and that deglutition was difficult. The mother stated that fluids often returned through the nose. The head lolled continually due to bilateral weakness and slight atrophy of the sternocleidomastoid and trapezius muscles. The tongue could not be protruded. These signs indicated involvement of the tenth, eleventh, and twelfth cranial nerves, perhaps through injury to the motor nuclei. There was loss of articulate speech due apparently to a persistent aphasia, as well as to impairment of phonation.

Case 3: A nine-year old girl (IK) from Heanza, who was examined on June 14, 1946, eleven months after onset, displayed a persistent aphasia, with previously adequate vocabulary reduced to *hai* ("yes") and *ie* ("no"). Examination was otherwise entirely normal and, except for the loss of speech, she appeared to be a normal healthy child.

Case 4: Another patient (TK) had undergone a prolonged convalescence with

disorientation as the dominating residual symptom, and was considered to have a persistent organic psychosis when eventually discharged on November 7, 1945, three months after onset. On examination ten months after onset, June 22, 1946, this fifteen-year old girl showed only a mild personality change, evinced by slight emotional instability, and was caring for the younger children in the family in an adequate manner.

Case 5: A four-year old male (KS) had been admitted to the hospital in coma on August 8, 1945. As the state of consciousness cleared, observers noted weakness of the right arm and leg, which progressed to an almost complete hemiplegia. He was aphasic during this period. These abnormalities eventually disappeared and on discharge, October 5, 1945, he displayed only what one observer described as "involuntary, almost athetoid movements of his right arm". A rise in complement fixing antibody titer from negative to positive (1:32) was demonstrated during the course of the disease.

When examined on June 22, 1946, ten months after onset, this bizarre gesture of the right arm was still present. When disturbed or apprehensive, the child placed his arm over the head with fingers extended, in what still seemed to be a rather involuntary movement. Strength and tendon reflexes were considered normal.

The remaining six patients with sequelae showed varying degrees of mental retardation, altered reflexes, focal motor weakness (usually in the extensor musculature of one extremity), or incoordination of fine movements, such as those used in writing. No sensory disturbances were observed. All six of these, as well as the 27 who were considered to have recovered completely, gave histories revealing gradual improvement and return of function over a period of several weeks or a few months. All of the children were in school, and the adults were pursuing usual occupations.

In addition to these neurological sequelae, several patients showed scars of decubitus ulcers and one showed a minute, opaque corneal scar, all mute evidence of the previous debilitating illness. Faint parallel, linear scars were visible over the brow, mastoids, or occiput, and along the cervical and thoracic vertebrae of many patients, where the so-called "acupuncture" incisions had been made by some member of the household in an attempt to relieve the headache and spinal rigidity.

COMMENT

In the Okinawan epidemic of 1945, 102 civilians had been diagnosed after comprehensive study as having Japanese B encephalitis. 26 of these died during the early stages of the disease. Among 38 of the survivors, neurological sequelae were found in 11 nearly a year later. It is not unlikely that the incidence of sequelae would have been lower if all of the 76 surviving patients had been examined, but in no case could it have been less than 14%. The frequency of sequelae in this small group appears, therefore, to be considerably higher than that reported by Japanese workers following the larger epidemics between 1924 and 1928, although comparison is difficult because of variations in reporting, in age

distribution, and in the criteria used for diagnosis. Kaneko and Aoki (5) reported an incidence of only 3.11% for all types of residual damage among 2,000 cases. One might reason that under peace-time conditions they saw many mild cases, while on Okinawa cases of this type were missed. This interpretation is difficult to maintain, however, in view of the fact that the case mortality in Japan, ranging from 49.6% to 75.2% in various epidemics (6), was much higher than that on Okinawa in 1945.

It is certainly true that on Okinawa and adjacent islands in the Ryukyus where outbreaks of varying magnitude apparently occur nearly every summer, the after-effects of the disease are not particularly evident in the population. In an American military government hospital which handled hundreds of Okinawan civilian casualties during the invasion of the island, one of the authors (T.W.S.) observed very few neurological or psychiatric abnormalities which could be construed as having been the result of any familiar neurotropic virus infection. Some Okinawan physicians were of the impression that, under the conditions of medical care that had existed, cases of *noyen*, or "summer encephalitis", as a rule either failed to survive or recovered almost completely. These facts suggest that the more adequate care given the selected group of cases had allowed the survival of some patients with more severe damage to the central nervous system.

SUMMARY

Examination of 38 individuals on Okinawa, in whom a diagnosis of Japanese B encephalitis had been made ten to twelve months previously, revealed that 11 had clinically detectable neurological sequelae. These included abnormalities of mental status and motor function. In only three patients was there a severely incapacitating disability.

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AEDES AEGYPTI CONTROL IN THE ABSENCE OF A PIPED POTABLE WATER SUPPLY

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In 1939, when British Guiana became a vitally important source of supply of bauxite, the health authorities of the Colony felt that drastic precautions should be taken to protect the air and ocean ports as well as other coastal communities against the introduction of yellow fever. Although yellow fever had not been known to occur in the thickly populated coastal areas of the country since 1909, investigations by Sneath in 1939 (1, 2) established the fact that the jungle form of the disease was present in the interior. An *Aedes aegypti* survey showed that the incidence of these mosquitoes in strategic areas was high.

On the invitation of the Government of the Colony the International Health Division of The Rockefeller Foundation agreed to assist the health authorities in an anti-aegypti campaign. A yellow fever control service was established, and anti-aegypti measures were begun in the fall of 1939. The work was directed first by Dr. A. W. Burke and later by Dr. George Bevier, both of the Foundation staff. I joined the Yellow Fever Control Service in 1942, spent six months in Brazil during 1944 under a Rockefeller Foundation fellowship, studying methods of yellow fever investigation and control, and was appointed chief officer of the Service in December 1945, when Dr. Bevier left British Guiana.

The classical *Aedes aegypti* control measures, as established in Brazil, were employed in British Guiana from 1939 to 1945. Personnel for this work included a staff of zone inspectors to make routine examinations, at 7-day intervals, of all water containers within and in the vicinity of houses, to insure absence of aegypti breeding; a marine inspection service to search for aegypti breeding foci on docks and ships; an adult mosquito capture service; squads to search for hidden foci and a squad to inspect high tanks and water boxes difficult of access. A strict system of supervision and revision was maintained, and it can fairly be claimed that a relatively high standard of efficiency prevailed. In rural areas *A. aegypti* were readily eradicated by the breeding control measures. In urban areas, however, particularly in the city of Georgetown, results were less satisfactory, for although aegypti house indices (*i.e.* the percentage of houses inspected in which adult mosquitoes or water receptacles containing pupae, larvae or eggs were found) became relatively low, these indices fluctuated with the rainfall and eradication was not achieved.

One of the main obstacles to success was the occurrence at times of a 4-day egg-adult cycle. Although such a short cycle was more the exception than the

¹ The British Guiana Yellow Fever Control Service, during 1939-46, was financed jointly by The Rockefeller Foundation, the Government of British Guiana and from a grant under the Colonial Development and Welfare Scheme in the West Indies.

rule, it occurred often enough to permit a certain amount of reinfestation within the 7-day inspection cycle, yet a universal 4-day inspection cycle proved neither practical nor economical. Another deterrent to success was the long delay in legal action against recalcitrant householders who refused to comply with the Yellow Fever Control Regulations. Pending the hearing of a charge against an offender the Yellow Fever Control Service could not take any action against this person.

Late in 1945 a small experiment was carried out to determine the extent to which *A. aegypti* could be controlled by spraying the interiors of houses with DDT. The prolonged residual action of this insecticide proved so effective that routine breeding-control measures were considerably modified during 1946.

The special mosquito-breeding problems encountered in British Guiana and the methods used to overcome them will be described in the following pages.

CONDITIONS CONDUCIVE TO MOSQUITO BREEDING

The city of Georgetown, with approximately 90,000 inhabitants, does not have a piped potable water supply, and for this reason the task of *Aedes aegypti* eradication there has presented great difficulties. Rain water is collected off the roofs by galvanized roof-gutters, and the water is led into large storage containers, such as vats, tanks, barrels and drums. Water is drawn from these, as required, and kept in the houses in every imaginable type of usual and unusual vessel. In some areas artesian well water is bought from vendors during the dry seasons and carefully hoarded, being very often hidden away from the prying eyes of inspectors.

Roof-gutters. In 1943 it was recognized that in Georgetown roof-gutters were probably the most dangerous source of mosquito breeding. Eggs laid in these gutters remained viable for months in the dry season and hatched out as soon as rain fell. It is considered that the main "carry over" from one wet season to another was effected in this way. "Flaming" gutters with a blow torch, to destroy eggs, could not be undertaken, as this would have created too serious a fire hazard since the houses in Georgetown are of wooden construction.

The very high temperatures which obtained in the gutters may have been responsible for the fact that the shortest egg-adult cycles were recorded for material from them.

The roof-gutters are often long and badly graded. Rotting fascia boards, to which they are attached, cause sagging. Overhanging trees shed leaves into them, and blockage results. Every conceivable type of refuse, including human and animal excreta, has been found in the gutters of sheds overlooked by windows. In the vertical sections of these gutters, blockage has been caused by cricket and tennis balls, leaves and even a 7-foot snake.

Large squads of men were organized to examine gutters on short inspection cycles. To speed up control work these men cleaned the gutters, did simple grading by means of wooden chocks and, with the householders permission, trimmed overhanging trees. Gutters where breeding was occurring were oiled. While this measure was not always successful in destroying larvae, it stimulated

the householder to take early action so as to avoid having the oil drain into the vat or tank. Notices were served to householders to rectify all major defects. But during the war years there was an acute shortage of materials for repair; and furthermore many householders neglected to make repairs that were possible. Thus a single gutter might remain a potential source of contamination for weeks



FIG. 1. TYPICAL LARGE HOUSE SHOWING ROOF-GUTTERS

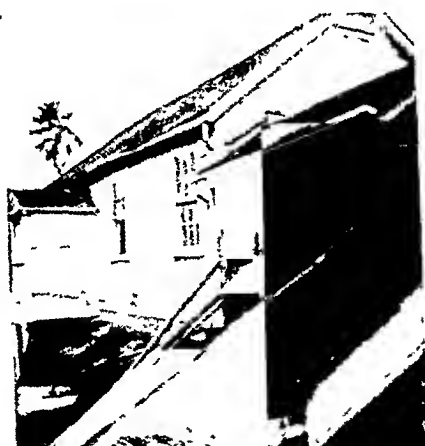


FIG. 2. LONG, BADLY GRADED ROOF-GUTTER

on end. In 1946, statutory authority was obtained to perforate defective gutters so that they would not retain water.

Containers for water storage. Large wooden vats or tanks, with an average capacity of about 2,000 gallons, can be found in almost every yard in Georgetown. Sealing of these is too expensive and short lived to be practical, and in order to control mosquito breeding in them five or six "silverbait" (*Tetragonopterus chalcus*) were placed in each one. The squads checked the presence of the larvivorous fish in the vats on each inspection visit, using breadcrumbs to coax them to the surface. Fish die if they are placed in new tanks during the first few weeks after these are put into use, probably because of the presence of toxic



FIG. 3. TREES OVERHANGING ROOF-GUTTERS



FIG. 4. INSPECTING A ROOF-GUTTER WITH A PERISCOPE MIRROR



FIG. 5. CLEANING A ROOF-GUTTER

products from the caulking material. Larvae, however, will live in these new receptacles.

Barrels and drums are widely used as water containers in some areas and, wherever present, they have been a constant source of trouble. They are too small to accommodate fish, which die from the heat. Sealing, which is a relatively simple matter, perishes quickly. A single small flaw in the sealing, often undetected, can cause serious trouble. Many householders delayed sealing such receptacles until they were brought into court—often a slow process—and the

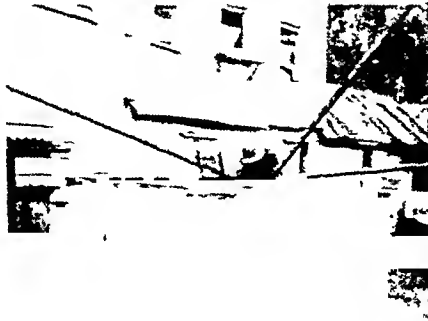


FIG. 6. LARGE WOODEN VAT



FIG. 7. DRUMS FOR WATER STORAGE

offending barrel and drum would contaminate the surrounding area. Eventually, to save time and money, the Service undertook the sealing of these containers; and although this was bad public health practice it paid large dividends. Drums without covers were marked by the Service, and unauthorised use of them for water storage constituted an offence.

Large zinc-lined water boxes, in which rain water was stored for bathing purposes, presented a similar problem. But since these were not exposed to the weather they could be sealed without fear that the sealing materials would deteriorate quickly.

Wash tubs. Small wooden tubs, for the washing of clothes, proved to be excellent test containers. These vessels cannot be left dry or the wood deteriorates; therefore water is kept in them when they are not in use, and the owner rarely changes the water daily as instructed. The absence of breeding in tubs is an excellent indication of the absence of *Aedes aegypti* on the wing. Oiling these



FIG. 8. WOODEN TUBS FOR WASHING CLOTHES



FIG. 9. CLAY CONTAINER AND ENAMEL BASIN HIDDEN UNDER A BED

containers brought forth considerable antagonism, as it requires much effort to render them clean and fit for use again.

Hidden mosquito producing foci. The hoarding of water, as a precious commodity, presents a serious problem in aedes control. The zone inspector has to use considerable tact and ingenuity to make a complete inspection of a house. Frequently water containers have been found hidden under beds and in out-of-the-way places; and more often than not they contained mosquito larvae.

As the *Aedes aegypti* house indices fell, the last few remaining producing foci were almost always of one of three types

- (1) Tree holes as high as 30 to 40 feet from the ground. Aedes were often found breeding in very dirty water in these holes, and this was interpreted

as Nature's effort to preserve the species. The holes were either opened up or filled.

- (2) Large dirty ground pools under the wooden floors of houses built close to the ground. Here again *Aedes aegypti* was not in its natural habitat, viz. clean water. This type of focus could be eliminated only by ripping up the floor and filling in the pools.
- (3) Improperly sealed barrels or drums



FIG. 10 TREE HOLES



FIG. 11. UNDER THIS WOODEN FLOOR A LARGE GROUND POOL WAS FOUND

Prevention of Reinfestation

Traffic between infested and clean areas by land, sea and air must be carefully watched. Road traffic did not seem to be a dangerous source of reinfestation, but occasional single female *Aedes aegypti* were, no doubt, imported in this way.

Trains were a serious problem. Pyrethium spraying at their point of departure for Georgetown reduced, but did not eliminate, the hazard. Treatment with DDT and Gammexane smokes had a brief but not a residual action. Spraying with 5 per cent DDT in kerosene solved the problem. It is advisable to spray all trains and railway stations.

Ocean-going ships rarely brought *Aedes aegypti* into port. Intercolonial schooners, trading between the neighbouring islands and British Guiana, were a constant source of reinfestation of the Georgetown dock area. These vessels were examined in mid-stream, and *Aedes aegypti* adults and breeding foci were eliminated by pyrethrum spraying and oiling of water containers. The Service sealed containers, on request, at the expense of the owner of the schooner.

River craft, except the larger steamers, were not often found to have breeding foci of *Aedes aegypti*. A careful watch over them is nevertheless essential, particularly for maintenance routine.

Discipline and Efficiency

Discipline of inspection squads must be strict. The prompt weeding out of the inefficient and unsuitable inspector is essential if *Aedes aegypti* eradication is to be regarded as a possibility. It has been our unfortunate experience, time and time again, that an unreliable inspector can cost the Service literally thousands of dollars and delay progress for weeks or even months. The process of constantly checking the work of the inspectors, discovering their deficiencies and bringing the inefficient to book, is a most unpleasant one. But it must be done, no matter what control methods are used, if aedes eradication is to be undertaken economically or successfully. Recognition of this fact is regarded as perhaps the most essential feature of an anti-aedes campaign.

Particularly when low indices have been attained, a bonus system, whereby a zone inspector can earn a monthly sum—in addition to, and separate from, his salary—serves as a constant incentive. It produces a higher standard of efficiency in the field and automatically singles out the incompetent inspector. These bonuses are based on efficiency ratings. Inspectors failing to reach a minimum rating on two or more occasions should be discharged. The time comes when a single missed focus will mean a zero rating. This may appear harsh, but it signifies that the inspector failed to find the one focus in his zone during that particular month!

LEGISLATION

The ideal procedure for quick results in aegypti control is the Brazilian system, whereby the officers of the Service deal with offenders directly and fines are collected through the legal department. Where the constant threat of a yellow fever epidemic is not present to stimulate public cooperation, and where the public has to be educated to accept aedes control, there will always be some persons who will not, or cannot, recognise the need for house inspections and the prompt rectification of any defects in water containers. For the common good, recourse to the law is essential in such cases. A system whereby non-cooperative householders can be promptly dealt with is an urgent necessity, unless much time and money are to be squandered.

Failing such a system the Service will find it cheaper in the long run to undertake the simpler repair jobs, *e.g.* trimming of overhanging trees, simple grading of roof-gutters and the sealing of barrels and drums, as part of the routine work.

DDT AND AEDES CONTROL

We have had experience in aedes control by means of house spraying with DDT in areas where no previous work had been done and in areas where work was in its final stages.

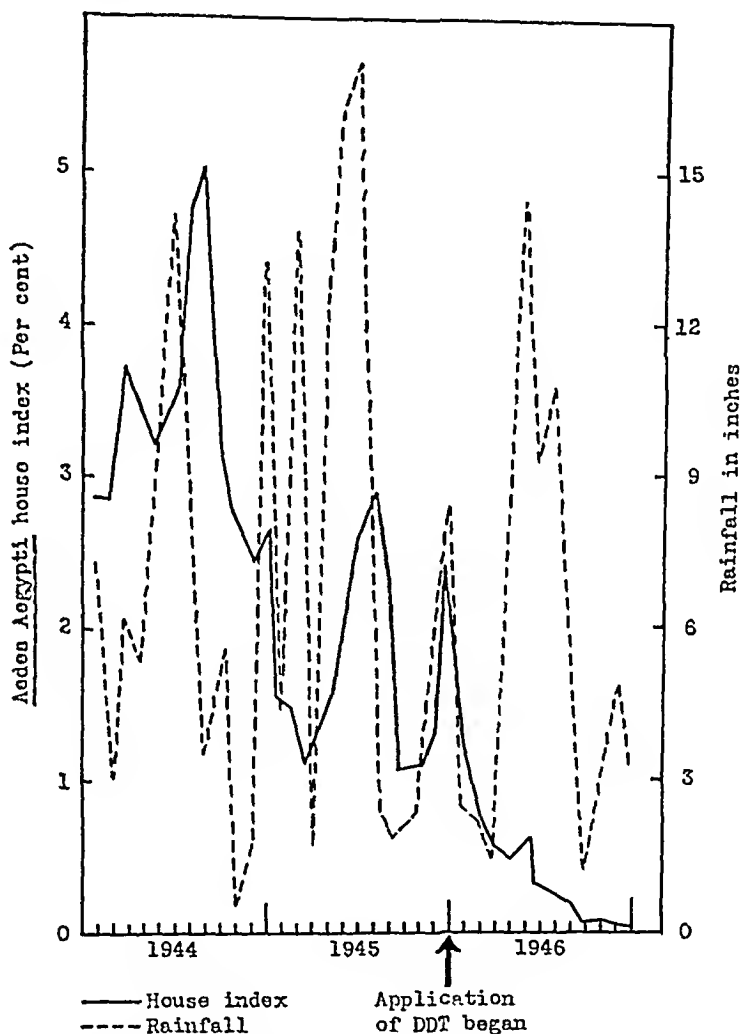


FIG. 12. THE MONTHLY TREND OF AEDES AEGYPTI HOUSE INDICES AND OF RAINFALL BEFORE AND AFTER THE APPLICATION OF DDT, GEORGETOWN, BRITISH GUIANA

A 5 per cent solution of DDT in kerosene— $\frac{1}{2}$ lb. technical DDT to 1 gallon commercial non-deodorized kerosene—was applied in an estimated dosage of 100 mgm. DDT per square foot to walls and low ceilings—at a cost of:

DDT	B.W.I. \$0.85 ² per lb.
Kerosene	B.W.I. \$0.3269 per gallon (both duty free)

² Cost is still unstable and varies between B.W.I. \$0.75 and B.W.I. \$0.95 per lb. U. S \$1.00 is equivalent to B.W.I. \$1 19875.

It has been possible, using power sprayers and "Cooper" hand pumps, to spray the average 3- to 5-room house at a cost of B.W.I. \$1.76 per house. Depending on the density of the population, this has worked out at between B.W.I. \$0.47 and \$0.59 per head of population.

Under this treatment previously unworked areas attain zero indices 11 to 13 weeks after spraying. Judged by appropriate control methods, a single spraying still remains effective at Plaisance after 17 months (3). The usual 8- to 12-week "carry over" from a large producing focus can be eliminated in two weeks by localized DDT spraying, often confined to a single house. Hidden producing foci can be effectively wiped out in this way, even when the location of the focus remains undiscovered, although this occurs but rarely.

Under conditions in the rural areas in British Guiana routine classical anti-aedes measures yield a zero index after about four months at a cost of B.W.I. \$1.18 per house and \$1.39 per head of population. In urban areas these figures are infinitely greater.

In our experience DDT has proved itself to be the method of choice for aedes eradication, particularly in areas without a piped potable water supply. It is cheaper and more rapidly effective than classical aedes control measures. By eliminating the personal factor, i.e. the efficiency or lack of efficiency of the inspector, it has solved the basic problem in aedes control. Reinfestation of clean areas as a result of negligence, as happened in a locality where a check survey yielded a house index of 16.1 per cent when zero indices were being reported, cannot occur.

In Figure 12 the trend of the aedes house indices in Georgetown is shown by month for the period 1944-46. Prior to 1946 anti-aedes measures failed to reduce the index below 1 per cent and fluctuations with rainfall continued to occur. When spraying with DDT was inaugurated these periodic fluctuations ceased and the index dropped steadily, reaching zero by the end of 1946.

In areas with a simple piped potable water supply system, the relative costs of classical anti-aedes methods and DDT spraying may vary but little. The additional advantages of the latter, viz., control of other disease-carrying mosquitoes and of various other insects, should be borne in mind. Furthermore, the DDT method eliminates much of the clerical work so necessary with the classical methods.

SUMMARY

The city of Georgetown, British Guiana, with its complex system of rain water collection and storage, in the absence of a piped potable water supply, presented an extremely difficult problem in *Aedes aegypti* eradication. An abnormally short egg-adult cycle added to the difficulties.

The special problems encountered and the methods used to solve them are described. The spraying of the interiors of houses with DDT proved a most potent weapon in aedes eradication and was cheaper and quicker than classical anti-aedes measures.

ACKNOWLEDGEMENTS

I am indebted to Dr. H. B. Hetherington, O.B.E., Director of Medical Services, British Guiana, for permission to publish this paper, and to the late Dr. Porter J. Crawford, of the International Health Division of The Rockefeller Foundation, for his interest and advice.

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ASYMPTOMATIC TOXOPLASMOSIS

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On October 22, 1945, a 29 year old mestizo Salvadorean who had been working on the Isthmus of Panama for a year, despondent over a love affair, slashed his wrists and jumped off a 40 foot cliff. Necropsy disclosed that death was due to trauma to the brain, spinal cord, and liver.

The heart weighed 350 grams and grossly appeared normal. In a section taken from the left ventricular wall was a cyst-like structure measuring 54 x 96 microns composed of closely packed fusiform bodies each measuring 1 x 5 microns (Figure 1). Two hundred and seventy parasites could be counted in one focal plane under oil immersion objective. Each tiny body contained a small basophilic nucleus and slightly eosinophilic cytoplasm. No distinct capsule was seen. There was no striation of the limiting membrane and no trabeculae divided the cyst. Some of the parasites appeared loosely dispersed at the periphery of the cyst but whether these had been extruded during sectioning or not, could not be determined with any degree of certainty. No inflammatory reaction was present in that portion of the heart surrounding the parasite. No parasites were found in 75 additional sections prepared from 5 other blocks of the heart. In some of these other sections a few small areas of myocardial necrosis and fibrosis were seen and there was a slight inflammatory reaction with infiltration of lymphocytes, plasma cells, and an occasional macrophage.

No parasites were found in 164 sections of brain or in sections of the pituitary, thyroid, lungs, liver, spleen, adrenals, kidneys, testes, and prostate.

Definite classification of these parasites on the basis of their appearance in histologic sections cannot be made with complete assurance at the present time. *Histoplasma capsulatum*, *Trypanosoma cruzi*, and *Leishmania donovani* can be ruled out quickly. *Sarcocystis*, *Encephalitozoon* and *Toxoplasma*, however, can often be differentiated only with great difficulty. The size of the pseudo-cyst and of its individual parasites, the absence of a true cyst wall with striated membrane, and the location in striated muscle indicate that the correct diagnosis is probably toxoplasmosis.

In table 1 are listed cases in which similar parasites were found incidentally and that did not appear to be responsible for any clinical symptoms.

In 1909, Darling (8) described a case of a 20 year old Barbadian negro who developed, while working on the Isthmus of Panama, a disease that was considered to be typhoid fever although several atypical features such as leucocytosis, a negative blood culture, equivocal Widal reaction and severe muscle pains raised considerable doubt as to the diagnosis. Trichinosis was suspected and a biopsy of the biceps muscle was taken. Parasites that Darling considered to

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be Sarcosporidia but which we reclassified tentatively (5) as *Toxoplasma*, were found within the muscle. A severe myositis was present. Since chorioretinitis is a feature of congenital toxoplasmosis it may be noted that the patient's vision was described as defective although the description of the ophthalmologic examination is too vague for adequate interpretation. Recently Syverton and Slavin (9) reported a similar case in which toxoplasmas were demonstrated in the gastrocnemius muscle of a 65 year old male ill of a disease resembling typhoid fever. An inflammatory reaction was present in the muscle. These two cases may belong in the group of adult typhus-like toxoplasmosis as described by Pinkerton and Henderson (10) but cannot be appropriately included in a table of asymptomatic toxoplasmosis.

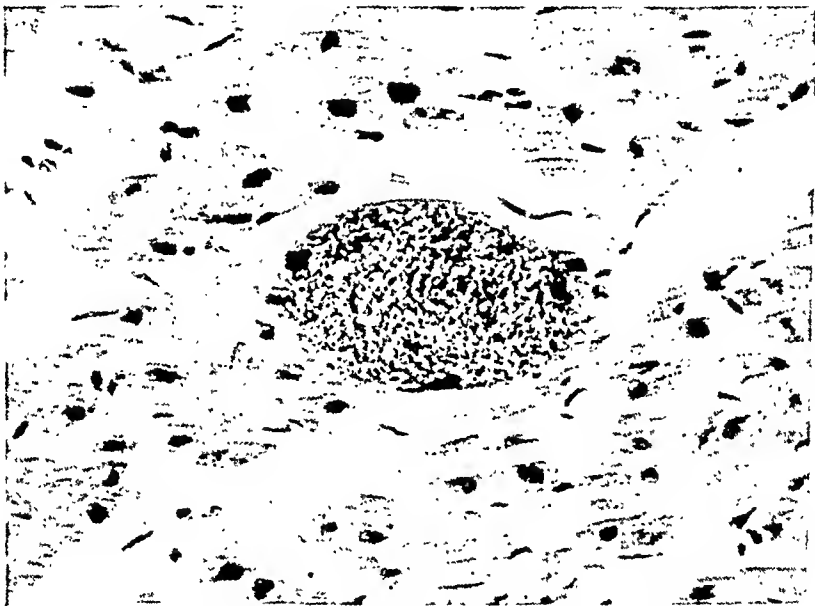


FIG. 1. CYST-LIKE AGGREGATE OF PARASITES IN CARDIAC MUSCLE. $\times 350$

In cases of congenital toxoplasmosis in which the infection in the fetus is widespread, destructive, and often fatal, the mother is apparently free of symptoms. It must be assumed therefore that the parasite can dwell in the human host without causing recognizable lesions. The site of predilection for parasites in fetal tissue is the brain, although almost any organ may be invaded. In these asymptomatic cases, however, the parasites have generally been found in the heart or skeletal muscle alone and attempts to demonstrate parasites in other organs were unsuccessful. Tomlinson (6), however, first found parasites in the brain of a 13 year old child who died of sickle cell anemia and then in a restudy of the heart found *Toxoplasma* pseudo-cysts.

The absence of an inflammatory reaction to the parasite is noteworthy. In most congenital cases an intense inflammatory reaction is present; however, in one premature infant who survived for 25 days after birth inflammatory changes

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EXPERIMENTAL SPOROTRICHOSIS IN MICE*

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The fungus disease sporotrichosis, in man, usually starts from a puncture wound of the hand, as, for example, from the thorn of a barberry bush. This primary lesion is followed by the development of caseous nodules, abscesses, or ulcers up the arm (1). Generalized forms of the disease are exceedingly rare.

This communication reports studies on these questions: (1) Can the mouse, an exceptionally convenient laboratory animal, be utilized for the production and standardization of experimental sporotrichosis as it has been for the production of experimental blastomycosis (2)? (2) Do several strains of the causative fungus produce the same experimental disease? (3) What is the nature of the experimental disease? (4) Can an experimental infection simulate the human infection of an extremity following inoculation by puncture?

Although experimental sporotrichosis has been produced by several investigators beginning with Schenck in 1898, there have been no clear answers to these questions (3).

MATERIALS AND METHODS

1. Organisms

Seven strains of *Sporotrichum Schenckii* were used (Fig. 1). Strains C, D, E, and G were supplied by Dr. Norman F. Conant of Duke University. The notes refer to growth at room temperature.

Strain A. This organism was obtained from Dr. Lee Howard of Savannah, Ga. The patient, a woman, had a fluctuant lesion on the dorsum of the hand, and a series of nodules extending to above the elbow. At one month, on glucose agar media, the culture was black, with cone-shaped growth above the surface.

Strain B. Also from Dr. Howard. At two weeks, on glucose agar media, the colonies were black and elevated, with subsurface growth.

Strain C. From epithelioma-like mass on hand of 15-year-old Indian boy at Huanta, Peru. At two weeks on glucose agar the culture was gray.

Strain D. From lesion on back of hand. Boston, Mass.

Strain E. From fluctuant abscess at elbow of 11-year-old girl in Duke Hospital, Durham, N. C. The ulcerated primary lesion of the thumb was accompanied by nodular and ulcerated lesions at intervals up the arm. Fig. 82 and 83B in "Manual of Medical Mycology" are from photographs of this case (1). At 12

* Much of this work was carried out in the Department of Pathology of Duke University School of Medicine. Aid from the American Foundation of Tropical Medicine, Inc., has been of assistance. A partial report appeared in Federation Proceedings, 1942, 1, 173.

days, on glucose agar, black corrugated colonies, with abundant subsurface growth, were present.

Strain F. From child, Pediatrics Service, Duke Hospital. At two weeks the cultures were heavily pigmented.

Strain G. From case in Boston, Mass., 1938. After two weeks on glucose agar colonies were grayish-brown. Heaped up umbilicated areas occurred, but no crinkly appearance. Subsurface growth was abundant.

2. Preparation of suspension

The fungus, grown on glucose agar at room temperature, was harvested by picking up the portion above the surface of the agar by means of a loop. The mycelium and spores thus obtained were suspended in physiological saline solution, centrifuged at 1600 revolutions per minute for 6 minutes, and then made into a 1 to 10 suspension. Gentle grinding in a mortar gave an even suspension.

TABLE 1
Number of mice inoculated with S. Schenckii and route of inoculation

STRAIN	INTRAPERITONEAL	INTO PAW
A	25	23
B	3	3
C	3	3
D	3	3
E	6	6
F	3	3
G	6	6
	52	47

3. Injection of suspension

One cubic centimeter of the suspension was injected into the peritoneal cavity when this route was used. When the suspension was inoculated into the paw, only 0.1 cc. would pass into the tight structures.

4. Mycologic and pathologic study

Material for culture on glucose agar was taken from lesions with care to prevent contamination. Complete autopsies were performed. It was customary to obtain blocks showing the point of inoculation in the abdominal wall, the abdominal wall elsewhere or the diaphragm, any abdominal nodule present, the liver, spleen, and lungs. In addition the testes, retrosternal lymph nodes, brain, heart, bones and joints were examined as seemed indicated. Similarly blocks were obtained from complete dissections of the mice injected intrapedally. Extra blocks of the affected extremity were obtained.

5. Number of mice employed

Table 1 shows the number of mice inoculated with suspensions of each of the

strains of the fungus. The inoculation was made into the peritoneal cavity or into a hind paw.

INTRAPERITONEAL INOCULATIONS

1. Survival of mice

The majority of mice were sacrificed at intervals to study the disease in various stages.

Of 16 mice that died spontaneously it seemed fair to say from autopsy that 6 died as the result of sporotrichosis at from 17 to 88 days following inoculation (17, 20, 23, 24, 81, and 88 days). One died of a combination of sporotrichosis and a chronic non-fungus process (20 days). Six died chiefly of some factor other than sporotrichosis, such as bacterial pneumonia or bronchitis (2, 8, 13, 18, 19, 36 days). The abdominal sporotrichosis may have predisposed to death from these other causes. Three died and were autolyzed to the extent that the cause of death could not be determined.

In terms involving fewer figures, then, it may be stated that sporotrichosis in the mouse was the cause of death in the period between $2\frac{1}{2}$ and $3\frac{1}{2}$ weeks, due to the extensive peritoneal and hepatic sporotrichosis, or in the later stages, at about 12 weeks, in connection with widespread lesions including sporotrichosis of the tail and extremities.

2. Gross lesions

At 5 days after injection a nodule was present in the anterior abdominal wall at the point of inoculation. The immediately underlying peritoneum showed one or more dark nodules. Intra-abdominal fatty tissues were sometimes adherent to this site. Other black nodules, 1 to 2 mm. in diameter, were adherent to the tissues between the spleen and stomach or along the left iliac vessels. All of the dark nodules represented the pigmented fungus originally injected. In addition there were thin whitish plaques on the surface of the spleen but usually not elsewhere.

At 9 days, flat nodules up to 2 mm. in diameter had appeared on the peritoneal surfaces.

At 14 days there were white nodules less than 1 mm. in diameter on the surface of the epididymis. In mice the tunica vaginalis is continuous with the peritoneal cavity and extension of infection to the serous cavity of the scrotum is to be expected.

At 17 days, caseous material occurred around the left testis, and white plaques were observed on the liver for the first time.

At 19 days, minute gray dots, less than 1 mm. in diameter, became visible on the free and cut surfaces of the liver.

At 20 days, the peritoneum continued to show nodules, and one in the upper abdomen measured 0.5 cm. in diameter. There were fine granulations on the diaphragm and tunica vaginalis and in the liver. The nodule at the point of inoculation was liquid centrally.

At 23 days retrosternal lymph-nodal involvement had developed. The retrosternal nodes drain the peritoneal cavity. Also, the nodule at the point of inoculation had ulcerated to the peritoneal surface. In one mouse the peritesticular sporotrichosis had ulcerated through the scrotum; and there was a sporotrichotic nodule in the tail, and a sporotrichotic ulcer on the skin over the knee. The nodule on the tail contained spindle-shaped forms of *S. Schenckii*.

Between the 29th and 31st day there was no perceptible change in the gross lesions of the mice inoculated with Strains A to F. A mouse which died spontaneously on the 31st day had swollen paws. Pus from the ankle region showed innumerable tissue forms of the fungus. There were abdominal nodules, diaphragmatic granulations extending to the pleura and, rarely, minute nodules in the liver.

At 38 days an emaciated mouse was found dead. Photograph (Fig. 6) shows the ulcerated scrotum and the swollen paws. Nodules occur along the tail. White nodules up to 0.5 cm. in diameter may be observed on the anterior abdominal wall, between the spleen and stomach and on the diaphragm. A thick layer of caseous and fibrotic material lies between the diaphragm and liver, and similar material fills the pelvis. A nodule lies on the surface of the heart. Preparations from the pelvic material and from a paw showed the fusiform fungus.

3. Microscopic appearances

Microscopically, at five days the brown spores of the original inoculum were seen in the nodule at the point of inoculation and in some of the scattered peritoneal nodules. Fragments of mycelium were visible. The originally inoculated material was in part dead and confluent. In addition, polymorphonuclear neutrophils had accumulated, and there was a layer of macrophages with underlying fibrosis (Fig. 2). The fusiform and oval fungus organisms were in part free, in part within macrophages. Lymphocytes were present in small numbers. Organisms were found, in some mice, in the Kupffer cells of the liver and in the phagocytic cells of the alveolar walls of the lung.

At 9 days, organisms were noted in the peribronchial lymph nodes.

At 13 days, a mouse showed much sporotrichosis of the peritoneum. The fusiform organisms were numerous, often within macrophages. Organisms occurred in the spleen, liver (Fig. 3), and lungs,—a true septicemia. The hepatic foci were clusters of macrophages containing organisms. The splenic lesions were in part necrotizing, but there were organisms within macrophages. Death of the mouse was apparently caused in part by pneumonia due to a gram-positive coccus. In the retrosternal lymph nodes the fungus was present in enormous numbers, usually free.

At 20 days, giant-cell reaction was noted in one mouse but this type of reaction was unusual.

At 23 days, phagocytosis of the fusiform organisms by macrophages was well-developed (Fig. 4).

At 24 days, one mouse showed in addition to peritoneal and hepatic lesions, a nodule within the substance of the tail which consisted of foci of necrosis in

the marrow with adjacent macrophages filled with the fungus organisms. In the abdominal cavity a nodule on the surface of the spleen had become necrotic centrally (Fig. 5).

At 81 days a mouse showed peritoneal, hepatic, pulmonary, pleural and myocardial sporotrichosis, and sporotrichotic lesions of the tail and all extremities as well.

A mouse at 88 days showed more fibroblastic encapsulation of the lesions of the abdomen and also fibroblastic foci in the liver and lungs suggestive of healed foci. There were lesions of the myocardium, but no organisms. Organisms persisted in the liver.

In a mouse sacrificed at 107 days organisms were still very numerous.

Strain G acted differently from Strains A to F. The lesions were not progressive. The peritoneal nodules were small, and no organisms could be demonstrated in them after the first few weeks. Moreover, extension to the liver and elsewhere never occurred.

4. The amount of inoculum

The dosage factor did not seem especially important. While 1 cc. of the 1 to 10 suspension was used in most mice, a 1 to 25 suspension was used in 5 mice which received Strain A, without discernible differences in the lesions produced.

5. The injection of human pus

Three of the six mice injected with strain E did not receive the suspension of culture, but received pus from the patient from whom the culture was grown. This pus, in which organisms could not be identified by direct examination, was diluted 1 to 10 with saline solution. Each of the 3 mice received 1 cc. of the diluted pus. The number of organisms in each injection must have been exceedingly small, but extensive sporotrichosis was produced in 2 of the mice. When they were killed 107 days after injection, both had abdominal lesions and one had hepatic lesions. The third mouse that received pus was killed 16 days after inoculation and no lesions were demonstrable grossly or microscopically.

6. Cultures from experimental lesions

In about half the mice, cultures were made from various lesions. Recovery of the organism in pure culture from nodules of abdominal sporotrichosis was possible in practically every instance in which it was attempted, except from nodules of mice inoculated with Strain G. The mice which received Strain G and from which positive cultures were obtained were examined 2, 8, and 37 days after inoculation, while those from which negative cultures were obtained were examined 35, 36, and 95 days after inoculation. This indicates that Strain G was viable at first and later died out. In those mice injected with other strains, however, the organisms remained viable until the time of examination, even when the examination was performed a very long time after injection. This applied to mice examined at the end of periods varying from 3 to 3½ months.

Positive cultures were obtained from various other parts of the body, as for

example, from the lesions of the tail and extremity. That a septicemia due to *S. Schenkii* may be present was demonstrated in 2 mice inoculated with Strain A and killed after 9 days. In one of these, positive cultures were obtained from brain and lung, and in the other from blood and spleen. These cultures were obtained with extraordinary precautions to prevent contamination from the fungus in the abdominal nodules.

7. Direct observation of organisms

Direct microscopic examination of fresh material from the lesions was about as convincing as the culture method in demonstrating the presence of the organisms. The pus or caseous material was examined in 10% sodium hydroxide solution, in lactophenol cotton blue solution, or in smears allowed to dry without heating, and stained by the method of gram.

INOCULATION INTO PAWS

1. Survival of mice

Experimental sporotrichosis of an extremity was not as serious a disease as sporotrichosis of the abdominal cavity. A few deaths occurred from generalization of the infection. Thus there were deaths after 61, 65, and 92 days which were attributable to sporotrichosis. In a few mice there was spontaneous recovery from the sporotrichosis of the extremity, after several months.

2. Gross lesions

The paw became tense when the inoculum was injected. This had subsided by the following day. At the end of a week the paw was found to be swollen. At two weeks the paw was greatly swollen and ulcerations were present. The effect of bilateral injections into the paws is shown in Fig. 7, with a normal mouse for comparison. The injection had been made $2\frac{1}{2}$ weeks previously.

Two months after inoculation the paws were less swollen, but the ankles had become enlarged. There had been discharge of pus from the swollen feet at intervals, and crusts had often been observed. The appearances $9\frac{1}{2}$ weeks after bilateral injections into the paws are shown in Fig. 8. A lesion of the tail is also present. The process caused a paw to drop off in one mouse at the end of 14 weeks.

The popliteal lymph nodes were enlarged during the period from 3 weeks to 2 months after injection.

The visceral lesions were not prominent grossly.

Strain G failed to produce swelling of the feet except in one mouse. In this mouse the swelling receded at the end of 33 days.

3. Microscopic lesions

Sections of the paw showed neutrophilic response with minute abscesses, caseation, and macrophages containing fungus organisms in the period from 5

days until 2 months and more. Osteomyelitis was produced, usually after 2 months, but in one mouse at two weeks (Fig. 9).

The popliteal nodes showed foci of necrosis and abscesses (Fig. 10). The fusiform fungus organisms were abundant, especially after two months, and were usually within macrophages. Scars in popliteal nodes had formed at 4 months, indicating healing.

Microscopic evidence of spread to viscera occurred in three mice at the end of 65, 92, and 111 days respectively. There were lesions in the peritoneal cavity, liver, and lungs; and in the brain in one mouse.

4. Injection of human pus

Three mice were injected with the diluted pus from human sporotrichosis as already mentioned in connection with the intraperitoneal injections. Moderate swelling of the feet occurred and the sporotrichotic lesions were less extensive, with fibrosis occurring earlier. A positive culture was obtained from the foot of one of these mice sacrificed at 107 days.

5. Cultures of experimental lesions

Cultures of the lesions of the extremity and popliteal node were regularly positive in mice inoculated with Strains A to F. Cultures from the viscera were positive in several mice, as previously indicated.

DISCUSSION

The studies indicate the usefulness of the mouse in the experimental production and study of sporotrichosis. In accessory experiments, guinea pigs and rabbits were found to be less readily infected, and are far less convenient laboratory animals. Rats can be used satisfactorily (4, 5).

Of seven strains of *S. Schenckii*, six produced the same active experimental disease. The seventh strain was less pathogenic. The cultural growth and the microscopic appearances (Fig. 1) of this strain were slightly different, and this strain should probably be reclassified.

Experimental sporotrichosis in the mouse is a chronic progressive disease, at times fatal and at times self-limited. It is characterized by an abundant proliferation of *S. Schenckii* in the animal, so that enormous numbers of organisms are present. This is in sharp contrast to the situation in the human, in whom organisms are so few in number that they are usually not seen on direct examination of pus or sections of lesions. The large numbers of organisms in the mouse soon come to lie in macrophages for the most part. The macrophagic (reticulo-endothelial) system of the liver takes up the parasites. In this respect experimental sporotrichosis in the mouse is like histoplasmosis in the human. Histoplasmosis has been called a cytomycosis. Sporotrichosis in the mouse can be given the same term.

The experimental disease produces neutrophilic and necrotizing or caseating lesions in addition to the macrophagic response. Widespread necrosis is some-

times produced where great quantities of organisms occur in masses of macrophages, as in the peritoneal nodules. Finally, fibrosis is produced.

The lesions tend to break down, ulcerate, and extend. Hematogenous extension from the peritoneal cavity to the lungs and elsewhere does occur but in a less conspicuous fashion than in experimental blastomycosis in the mouse. The common hepatic lesions occur via the portal venous drainage of the infected peritoneal cavity. The lesions which developed in the tail and extremities of mice injected intraperitoneally were probably produced by hematogenous spread. On the other hand, they may have been produced by infection from the litter in the cage, since ulcerated scrotal lesions were present in some of the mice.

The experimental infection of an extremity by *S. Schenckii* has many features in common with the spontaneous human disease. The infection begins with a puncture wound and lesions higher up the limb develop. There is a tendency for ulceration. In the human the process spreads along the lymphatics. This feature is not obvious in the mouse, though the lymphatics may play a part. The regional lymph nodes are moderately enlarged in the mouse, and are abscessed, but they do not ulcerate through the skin. In the human, regional lymph nodes are said to be involved little or none. However, they are usually not susceptible to study by removal and section, as in the mouse, and inconspicuous lesions may be present. The experimental disease may be self-limited, as may be the human disease. The experimental disease of an extremity leads to generalized dissemination more frequently than does the human disease.

SUMMARY

1. Chronic progressive sporotrichosis of an extremity, rather like human sporotrichosis, was produced in the white mouse by a single injection, into the paw, of a suspension of *Sporotrichum Schenckii*, or of pus from human sporotrichosis. Such injections were made in 47 mice. Massive sporotrichosis of the foot and later of the ankle developed. The process lasted about two months and some mice recovered spontaneously. Several mice died from generalization of the disease after two and three months.

2. Intraperitoneal injections of the same inoculums resulted in peritoneal sporotrichosis which spread to the liver and sometimes elsewhere, as to the lungs, pleura, tail, and extremities. Intraperitoneal injections were made in 52 mice. Death was produced by the sporotrichosis in many mice at about 3 weeks or at about 12 weeks.

3. The experimental disease, whether of extremity or peritoneum, was characterized by the growth of enormous numbers of the tissue form of the organism, especially within macrophages. It was thus a cytomycosis, like human histoplasmosis. The abundance of organisms in the lesions of the mouse is in contrast to the paucity of organisms in human lesions. The lesions were suppurative, necrotizing and fibrosing, as well as macrophagic.

4. Six strains of *Sporotrichum Schenckii* from human cases of sporotrichosis in various parts of the world produced identical lesions in the mouse, while a seventh strain was only feebly pathogenic.

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PLATE I

Fig. 1. Seven strains of *Sporotrichum Schenckii*, A to G, from human cases of sporotrichosis. Cover-slip culture preparations grown on glucose agar at room temperature and photographed at the same magnification. Note the similarity of strains A to F, which produced identical experimental lesions, and the divergencies in morphology in strain G, which was much less pathogenic for mice.

PLATE I



PLATE II

Fig. 2. Peritoneal sporotrichosis, 6 days after intraperitoneal inoculation. Neutrophils at top, intermediate zone of macrophages, lower zone of fibroblastic repair, striated muscle at bottom. At this stage the organisms are in part free, in part within macrophages.

Fig. 3. Sporotrichotic nodules in liver, 13 days after intraperitoneal inoculation. The pale areas consist of macrophages containing the tissue forms of *S. Schenckii*. The infection has come from the peritoneal cavity by the portal vein.

Fig. 4. Fusiform and oval tissue forms of *S. Schenckii* in peritoneal nodule 23 days after intraperitoneal inoculation. Some organisms are free, but most were within macrophages. Gram stain.

Fig. 5. Peritoneal nodule attached to spleen (right), 24 days after intraperitoneal inoculation. The nodule is composed of macrophages containing the tissue forms of *S. Schenckii*. The central portion of the nodule is necrotic.

PLATE II

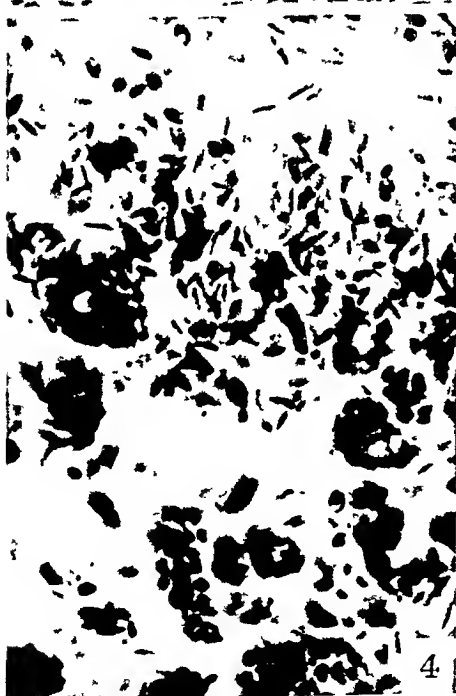
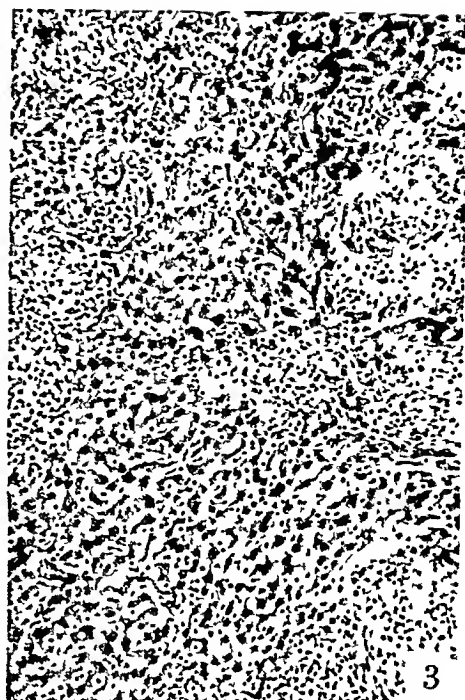
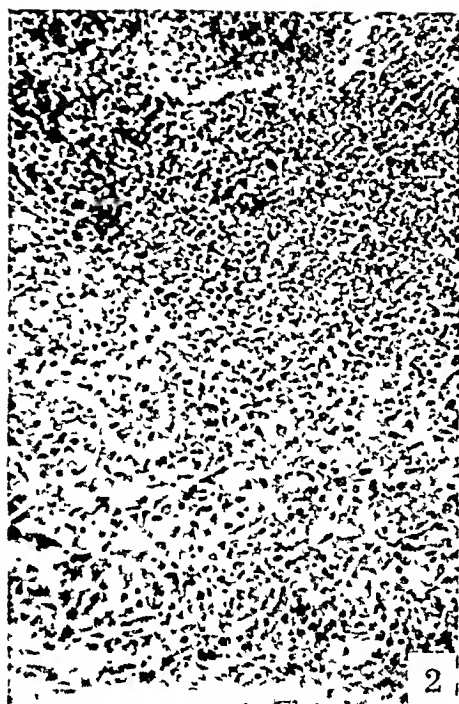


PLATE III

Fig. 6. Experimental sporotrichosis, 88 days after intraperitoneal inoculation of *S. Schenckii*. White nodules occur on the reflected peritoneum of the abdominal wall, on the under surface of the diaphragm, near the spleen, and in the pelvis. Ulceration of the scrotum has developed. A nodule lies on the surface of the heart. The swollen paws and the nodules of the tail are due to sporotrichotic lesions.

PLATE III



PLATE IV

Fig. 7. Ulcerating sporotrichosis of the feet, two and one-half weeks after bilateral inoculation of a suspension of *S. Schenckii* into the hind paws of the mouse on the left. The mouse on the right is a normal control.

PLATE IV



PLATE V

Fig. 8. Sporotrichosis of the hind legs nine and one-half weeks after bilateral inoculation of a suspension of *S. Schenckii* into the hind paws. The swelling of the paws has receded to some extent but the ankles have become swollen. The dark areas are crusted ulcers. The popliteal lymph nodes, which do not show, contain sporotrichotic lesions. Note the sporotrichotic nodule on the tail.

PLATE V

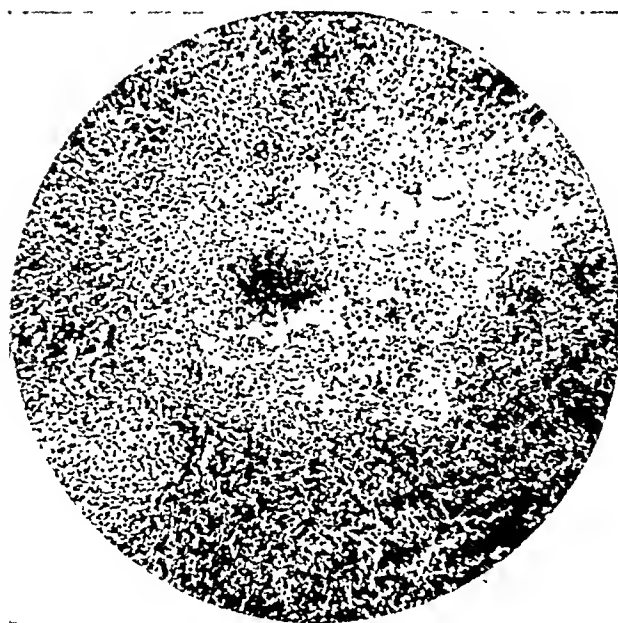


PLATE VI

Fig. 9. Sporotrichotic abscess in bone of paw following inoculation of *S. Schenckii*.

Fig. 10. Sporotrichotic abscess with adjacent necrosis and fibrosis in popliteal lymph node 10 weeks after inoculation of a suspension of *S. Schenckii* into the hind paw. The fusiform fungus organisms are abundant within macrophages in and about the abscess, but cannot be seen in the photograph.

PLATE VI



BENZENE HEXACHLORIDE FOR AREA CONTROL OF TROMBICULID MITES¹

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Scrub typhus, a mite-borne disease endemic over much of the far eastern and southwestern Pacific areas, was first encountered by American military forces at Port Moresby, New Guinea, in October 1942. With expanding military operations in the Pacific theater, scrub typhus rapidly developed into a medical problem of major proportions. Studies on mite repellents, which had been conducted for a short time in Orlando, Fla., in 1942 (Madden *et al.* 1944), were continued in 1943 and resulted in the development of effective methods for protecting individuals by impregnation of clothing (Snyder and Morton 1946).

Members of the Subcommittee on Research and Development of the Army Committee on Insect and Rodent Control pointed out that the heaviest infection of scrub typhus occurred in the first 48 hours of a landing operation, and that area treatment to supplement personal protective measures was imperative in virgin areas. Chemical control was considered the most feasible. Studies were begun in May 1945 at Orlando to determine methods of eradicating mites in their natural habitat. In preliminary tests of about 75 chemicals, selected primarily for their known insecticidal or fumigating properties, the outstanding compound was benzene hexachloride (Linduska *et al.* 1947). Additional tests of this material, some under practical conditions, provide the basis for this report. The predominant species of mites in the types of habitat included in these tests were *Acariscus masoni* Ewg. and *Eutrombicula alfreddugesi* (Oud.).

METHODS

The procedures were essentially the same as those used earlier in elimination tests for mite toxicants (Linduska *et al.* 1947). Mite populations were determined by exposing in plots of various sizes, for timed intervals, 5- by 8-inch black filing cards or Pyralin cards. The population index was obtained before treatment of the plots and at various day intervals thereafter. Control was measured by comparison with the pretreatment count. Counts made on six untreated check plots throughout the period of study (July–September, 1945) served to reveal any marked fluctuation in populations or activity. In general these counts varied little. However, marked fluctuations were observed on several days, and these are noted under discussions of specific tests in operation at the time.

¹ This work was conducted under a transfer of funds, recommended by the Committee on Medical Research, from the Office of Scientific Research and Development to the Bureau of Entomology and Plant Quarantine.

² Acknowledgment is made to E. F. Knipling, formerly in charge of the Orlando, Fla., laboratory, for suggestions regarding this study, and to S. R. Pratt and L. M. Wilce for assistance in field work.

SMALL-SCALE TESTS WITH VARIOUS FORMS AND DOSAGES

Two series of tests were made with benzene hexachloride. In the first series 1/1,000-acre plots were treated with a spray in cyclohexanone, or as a dust in talc or in the form of the crude compound undiluted, at dosages of 50 and 25 pounds per acre.³ The results in table 1 show that, when the material was applied as a spray or undiluted as a dust, several days were required before complete control was obtained, whereas the same dosages applied as a dust in talc were fully effective by the following day. The plots involved in this series of tests were covered by a dense stand of vegetation, 2 to 3 feet tall, and the more rapid action of the diluted dust may have been due to a better coverage and penetration to the ground level.

TABLE 1

Control of mites obtained in 1/1,000-acre plots with benzene hexachloride applied in various forms and dosages

TYPE OF APPLICATION AND DILUENT	CRUDE BENZENE HEXACHLO- RIDE		NUMBER OF MITES BEFORE TREATMENT ¹	PER CENT CONTROL AT INDICATED TIME AFTER TREATMENT				
	Concentra- tion (Per cent)	Dosage (Pounds per acre)		1 day	2 days	4 days	8 days	15 days
Sprays: Cyclohexanone	25	50	187	89	87	84	99	99
		25	126	83	83	85	98	99
Dusts: Talc	25	50	118	96	97	99	99	99
	10	25	159	98	97	97	99	100 ²
No diluent	100	50	216	86	86	93	99	99
		25	131	79	82	80	97	98

¹ Total mites counted on 8 cards exposed for 1 minute.

² 99 per cent control after 30 days.

In the second series of tests benzene hexachloride was applied on 1/250-acre plots, as a spray in cyclohexanone and in fuel oil and as a dust in talc. The dosages ranged from 6 to 20 pounds of toxicant per acre. Table 2 gives the results of these tests. Sustained rains (total precipitation 9 inches) for 2 days after the treatments prevented the taking of counts until the 4th day. However, counts taken between the 4th and 21st days showed practical control when a 3-per cent solution (approximate saturation) in fuel oil was applied and the dosage of toxicant was 6 pounds per acre. The application of 10 pounds of toxicant per acre, either as a spray in cyclohexanone or as a dust in talc, also gave almost complete control for at least 3 weeks.

Between the 10th and 16th days of testing a marked general increase in the

³ Dosages throughout this paper are for crude benzene hexachloride, reported by the manufacturer to have a gamma-isomer content of about 12 percent.

mite numbers or activity was indicated by a threefold increase in check-plot counts. Although most of the test plots did not reflect this population increase, the control dropped from 99 to 85 per cent in the plot treated at 6 pounds of toxicant per acre. A count in this plot on the 21st day showed that mites had again been reduced to a point of practical control (96 per cent). In other studies conducted during the same 21 days, a plot treated with a heavy dosage of benzene hexachloride showed a temporary increase in mites coincident with the general rise in counts in check areas. The sudden appearance of mites in this plot lowered the calculated control from 100 per cent on the 10th day following treatment to 17 per cent on the 16th day, but the control was again nearly complete several days later. Chigger counts in check plots continued near the high level during this period.

TABLE 2

Control of mites obtained on 1/250-acre plots with benzene hexachloride applied at graded dosages as sprays and as dusts

TYPE OF APPLICATION AND DILUENT	CRUDE BENZENE HEXA- CHLORIDE		NUM- BER OF MITES BEFORE TREAT- MENT ¹	PER CENT CONTROL AT INDICATED TIME AFTER TREATMENT					
	Concen- tration (Per cent)	Dosage (Pounds per acre)		4 days	5 days	7 days	10 days	16 days	21 days
Dusts: Talc	25	20	119	99	100	98	100	100	95
	10	10	127	100	98	99	97	99	98
Sprays Cyclohexanone	10	20	84	100	100	100	100	99	100
	5	10	66	100	97	98	95	98	95
No. 2 fuel oil	3	12 (50 gal.)	103	100	100	100	99	90	83
		6 (25 gal.)	95	99	99	100	99	85	96

¹ Total mites counted on 18 cards exposed for 1 minute.

From these observations benzene hexachloride in dosages as low as 6 pounds per acre has sufficient residual toxicity to kill newly emerged or invading mites for at least 2 weeks following its application.

The results of these tests are shown in table 3. The dust treatment, with 10 pounds of benzene hexachloride per acre, gave over 90 per cent control for 30 days. The 5-pound dosage gave a good initial kill, but control dropped below 75 per cent after the 12th day and continued to decline from that time on. The sprays appeared to be somewhat more effective on these larger plots, in which the vegetation was much less dense than in the 1/1,000-acre plots used in earlier tests. A 3-per cent solution in fuel oil applied at the rate of 6.4 pounds of crude benzene hexachloride per acre maintained a high degree of control for a full month, and appeared to be almost as effective as the 10-pound dust application. However, at a dosage of 2.5 pounds per acre the same spray gave an incomplete

initial kill and failed to provide acceptable control during the month of observation. Sulfur was relatively ineffective and even at the heavy dosage of 100 pounds per acre gave less control than benzene hexachloride applied at the rate of only 2.5 pounds per acre.

AIRPLANE TESTS

Preliminary tests were made to determine the feasibility of controlling mites with solutions of benzene hexachloride dispersed from airplanes. The test area was in a lake-edge type of habitat covered with a dense growth of mixed grasses and sedges 14 to 24 inches tall. A population index in 2 half-acre plots was obtained by exposing cards at 13 permanently marked sites in each plot. Fifty cards exposed on 5 successive days before spraying gave average counts of 158 mites in 1 plot and 118 in the other, or 2 to 3 mites per card-minute exposure.

TABLE 3

Control of mites on 1/5-acre plots with benzene hexachloride applied as dusts and as sprays at various dosages

TREATMENT	DOSAGE OF ACTIVE IN- GREDIENT (POUNDS PER ACRE)	DOSAGE OF MIXTURE (LR. PER ACRE)	AVERAGE NUMBER OF MITES BEFORE TREATMENT ¹	PER CENT CONTROL AT INDICATED TIME AFTER TREATMENT								
				1 day	2 days	4 days	8 days	12 days	16 days	20 days	30 days	
Dusts:												
Benzene hexachlo- ride, 25% in talc	10	40	234 ²	97	97	96	99	99	94	96	91	
Pure talc (control)	5	20	107 ³	93	93	86	79	77	65	43	48	
Sulfur, wettable	0	50	34	0	15	38	24	0	0	0	0	
	100	100	219 ²	61	53	67	43	16	18	7	0	
Sprays:												
		Gal.										
Benzene hexachlo- ride, 3% in No. 2 fuel oil	6.4	25	243 ³	93	94	92	96	92	95	94	89	
No. 2 fuel oil (con- trol)	2.5	10	62 ³	74	87	73	77	56	53	56	0	
	0	50	44 ³	61	61	70	68	52	37	5	0	

¹ Total mites counted on 25 cards exposed for 1 minute.

² Average for counts taken on 2 previous days.

³ Average for counts taken on 3 previous days.

A mixture of 12 per cent of benzene hexachloride in cyclohexanone was applied to the plots from a Stearman (PT-17) plane. The spray was delivered through ten $\frac{1}{8}$ -inch pipe outlets and under a pressure of 60 pounds per square inch. This fast-delivery apparatus released approximately 50 gallons per minute.

The planned rates of application on the two plots were 10 and 20 gallons per acre, or about 10 and 20 pounds of toxicant per acre. However, a heavy cross wind increased the anticipated swath width considerably, so that the actual dosages reaching the plots were equivalent to about 6 and 13 pounds per acre.

The sprays gave immediate and residual control of mites. Counts taken an hour after treatment showed complete kill or immobilization of mites on the plot

treated with 20 pounds per acre, and 94 per cent kill or knock-down with half that amount. Control in both plots was essentially complete during the entire 30-day period of observation. Counts taken up to a distance of 60 feet on the downwind side of the marked plots also indicated a complete kill, and further indicated the extent to which the spray had drifted.

Fumigating Action of Benzene Hexachloride.—Slade (1945) has demonstrated that benzene hexachloride exerts a potent fumigating action against the granary weevil (*Sitophilus granaria* L.), and recent tests at the Orlando laboratory have shown it to be similarly effective against mosquitoes and houseflies. In the present tests also the high efficiency of the compound against mites may have been due in large measure to a fumigating action. Preliminary laboratory tests to determine the mode of action were inconclusive. It was presumed, however, that if the material operated in any appreciable measure as a fumigant against mites it would give effective control without uniform coverage. A series of tests was therefore conducted in which benzene hexachloride was applied in different ways to several 1/1,000-acre plots on the same day. The material was applied as 50-per cent dusts in talc and as 25-per cent sprays in cyclohexanone at dosages of 50 and 100 pounds of benzene hexachloride per acre. On one group of plots the material was applied uniformly over each plot. In another group it was placed on areas of about 1 square foot at the four corners and the center of each plot. In a third group of plots the material was placed only at the four corners. Seven of the treated plots were checked on the day after treatment, and nearly complete kill was obtained on all plots regardless of the method of application. Counts on the remaining plots were delayed by rains, but examination on the third day showed no difference in control between plots.

Obviously there was some question as to whether the kill was due to fumigating action, or simply indicated a movement of mites such as would bring them in contact with the treated segments of the plots. However, all the plots had a stand of vegetation dense enough to retain fumes, and also a ground covering of duff and litter thick enough to restrict movement of the mites. These facts, together with the speed and thoroughness with which the plots were freed of mites, suggest that a toxic gaseous product was largely instrumental in control.

In another series of tests benzene hexachloride was impregnated as a 20-per cent acetone solution in 1-inch sections of $\frac{1}{4}$ -inch rope, in rabbit-chow checkers, and in sawdust. Sufficient amount of the carrier was used to absorb given amounts of the acetone solution. The impregnated carriers were then broadcast over plots. All the materials, when impregnated to give a dosage of 50 pounds of toxicant per acre, gave a kill equal to that obtained in previous spray tests in which the same volume of the material was applied uniformly over the plot. In each case immobilization or kill was complete within $2\frac{1}{2}$ hours, and 96 to 99 per cent after 10 days. Sawdust treated with the solution, dried, and then applied at rates of 20 and 40 pounds of toxicant per acre, gave complete kill within 24 hours and 98 and 99 per cent, respectively, after 4 days. Rabbit-chow checkers similarly treated and applied at dosages of 40 and 20 pounds of toxicant per acre gave practically complete control by the fourth day. The kill on the

first day was almost complete (95 per cent) with the 40-pound application but only 78 per cent at the 20-pound dosage. In other tests using this method of treatment at a rate of only 10 pounds of toxicant per acre a 92-per cent reduction was obtained within an hour. A much denser vegetative cover in the area of these tests is believed to have provided conditions which permitted more effective fumigating action.

Speed of Action of Benzene Hexachloride.—From a military standpoint the speed with which mites are eradicated in mite typhus areas is an important consideration. For use in invasion areas, military specifications called for a control material which would allow for safe entry immediately after its application.

In numerous tests with benzene hexachloride the kill was found to be high on the day after treatment (usually 18 hours). A few tests, with heavy dosages of 25 and 50 pounds of the crude material per acre, showed complete control of mites within $2\frac{1}{2}$ hours. A few additional tests with more practical dosages served to establish the minimum time limits of effectiveness of benzene hexachloride. Sawdust and rabbit-chow checkers were saturated with a 20-per cent solution of benzene hexachloride in acetone. After the acetone had evaporated, enough of the carriers were distributed by hand over 1/1000-acre plots to give a dosage equivalent to 10 pounds of toxicant per acre. In plots treated with the material in sawdust no mites were taken by the usual census procedure after 15 minutes, although an average count of 201 mites per 8-card exposure had been obtained immediately prior to treatment. Similar results were obtained 30 minutes and an hour later, and on the second day. The treated rabbit checkers appeared to require a slightly longer period to immobilize the mites. Control in this case was only 18 per cent 15 minutes after application, but 92 per cent after 30 and 60 minutes.

SUMMARY

Crude benzene hexachloride (gamma-isomer content of about 12 per cent) was found to be highly effective in the field control of the mites *Acariscus masoni* Ewg. and *Eutrombicula alfreddugesi* (Oud.). Practical eradication was obtained by applying this material as a 3-per cent spray in fuel oil, using about 6 pounds of the crude material per acre, and as a 10-per cent dust in talc, at the rate of 10 pounds per acre. A 12-per cent spray in cyclohexanone dispersed by airplane gave immediate and residual control at dosages of 6 to 13 pounds of benzene hexachloride per acre. Control was nearly complete within an hour and remained so for at least 30 days.

Preliminary field tests indicated that benzene hexachloride is effective against mites largely as a fumigant. This characteristic of the material, which was found to permit good control without uniform coverage, would also appear to adapt it for use against infestations in areas of tall, heavy herbaceous growth.

Complete control of mites was obtained by hand broadcasting of coarse materials, such as rabbit-chow checkers, sawdust, and small pieces of rope, which had been impregnated with benzene hexachloride in acetone solution. The dosage

necessary for control by this method was greater than by other methods, and appeared to be strongly regulated by the nature of the ground cover. In areas covered by a dense vegetative growth a rapid and practical control was obtained when as little as 10 pounds of the toxicant per acre was used. However, in plots with moderate ground cover dosages of 20 to 40 pounds per acre were required for the satisfactory elimination of mites.

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BYLAWS OF THE FOURTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA, WASHINGTON, D. C.,

MAY 10-18, 1948

PREPARATIONS FOR THE CONGRESSES

SECTION 1. BASIC ORGANIZATION

The Congresses shall be held under the sponsorship of the United States Government through its Department of State and Government agencies having an interest in tropical medicine in collaboration with the private societies composing the Intersociety Committee. These organizations shall nominate to the Secretary of State representatives for appointment to the Organizing Committee of the Congresses which shall be responsible for the preparations for the Congresses in cooperation with the Department of State. The Secretary of State shall name the Chairman of the Organizing Committee upon the recommendation of the cooperating organizations. The Organizing Committee may elect an Executive Committee from among its members and with the approval of the Secretary of State may select an executive secretary and such other officers as may be necessary.

The Treasurer of the Intersociety Committee shall be appointed in conformity with Section 4.

SECTION 2. DEPARTMENT OF STATE

The cooperation of the Department of State shall extend to the supervision or organizational and administrative arrangements including the issuance of invitations to governments together with informational material, certain financial assistance, documents, personnel, contacts and cooperation with other governments, official entertainment, hotel and travel reservations, meeting places, exhibit halls, and the necessary liaison with municipal authorities and others in the city which is chosen for the meeting of the Congresses.

SECTION 3. INTERSOCIETY COMMITTEE AND ORGANIZING COMMITTEE

The Intersociety Committee shall be responsible for the financing of the Congresses, except with respect to specific items which shall be the responsibility of the Department of State.

The Organizing Committee shall be responsible for the program including papers and speakers, nonofficial entertainment, professional invitations, exhibits, and the organization of tours of scientific institutions or other institutions of professional interest to delegates and members.

SECTION 4. COMMITTEES

To assist in the organization and conduct of the Congresses, the Organizing Committee shall select the personnel of the following committees and activate them:

Committee on Program

Committee on Entertainment (with Ladies Entertainment Subcommittee)

Committee on Publicity (later if needed)

Committee on Exhibits

Each Committee shall consist of a Chairman, Vice Chairman, Secretary, and the number of members necessary to carry out effectively the tasks assigned to it. These may be selected by the Organizing Committee from its own members or from the membership of any of the collaborating societies or organizations. All Committees shall work in close liaison with representatives of the Department of State assigned to different activities.

The Committee on Finance of the Intersociety Committee shall include a Chairman and Treasurer appointed by the Intersociety Committee.

MEMBERSHIP, FEES, AND BUDGET

SECTION 5. CLASSES OF MEMBERSHIP

The Congresses shall be composed of the following classes of participants:

1. Official Delegates
2. Institutional Delegates
3. Members
4. Associates
5. Sustaining Members

SECTION 6. FEES AND ADMISSION

The fees for professional members (other than official delegates who shall not pay any registration fees), for sustaining members, and for associate members shall be fixed by the Intersociety Committee. On payment of the requisite fee for any membership class, the Treasurer shall issue to such member an appropriate receipt. Proper notification shall be given to the conference registration officer so that a membership card or badge may be issued. Exhibition of such card or badge may be required as a condition of admission to general sessions or sectional meetings, to exhibits, or to entertainment or excursions.

SECTION 7. FEES AND BUDGET

The fees for any class of membership or any other financial contributions shall be paid to the Treasurer of the Intersociety Committee who shall issue receipt therefor. The Treasurer shall render monthly financial reports, and

also such interim reports as may be required, to the Chairman of the Intersociety Committee.

The Executive Secretary and the respective Committees shall submit to the Intersociety Committee budgets estimating (a) their routine operating requirements, and (b) amounts required to discharge their functional assignments. The Intersociety Committee shall, as soon as in their judgment sufficient funds have accumulated with the Treasurer, determine the budget allocation to each Committee for routine requirements; and not later than three months before the Congresses determine the budgetary allocation to each Committee for its functional activities. Committees shall not be authorized to incur expenses or liabilities in excess of their approved budget. Accounts payable of expenses incurred by a Committee shall, supported by appropriate vouchers, be certified by the Committee Chairman to the Executive Secretary, who, upon ascertaining from his records that it neither exceeds the budget nor the available balances, shall request the Treasurer to draw a check on the Congresses' funds in payment. The Organizing Committee and the Intersociety Committee sitting together shall from time to time review the financial position of the treasury and the Committee budgets, and effect such revisions as circumstances may require.

SECTION 8. PROFESSIONAL MEMBERS (INCLUDING DELEGATES)

Professional members shall have all rights and privileges of the Congresses, including, on presentation of credentials, admission to all general and sectional meetings, presentations of papers subject to selection by the Committee on Program, participation in discussions, the privilege of voting on questions coming before the Congresses, and shall be entitled to receive without further charge a copy of the final printed proceedings of the Congresses.

Persons coming within the following categories shall be eligible to become professional members:

Official Delegates: Official representatives of governments.

Institutional Delegates: Representatives of invited universities, societies, and scientific and philanthropic organizations interested in tropical medicine.

Members: Physicians, scientists, and other professional persons qualified in tropical medicine.

SECTION 9. SUSTAINING MEMBERS

Sustaining members shall be those persons, firms, corporations, or organizations which may choose to make at least the minimal contributions set by the Organizing Committee toward the financing of the Congresses on account of their interest in the furtherance of tropical medicine and hygiene. Such members may be given the right to present at the Congresses commercial exhibits provided such exhibits meet the ethical standards of the American Medical Association.

SECTION 10. EXHIBITS

Delegates and professional members shall have the privilege of displaying scientific exhibits presenting the results of individual or organizational con-

tributions to research or disease control on application to the Committee on Exhibits, provided such exhibits meet with the approval of the Committee and conform to such standards as may be prescribed.

Applications for assignment of commercial exhibit space shall be made to the Committee on Exhibits, which shall determine that the proposed exhibit meets with the standards referred to in Section 9 and any other just and equitable standards which the Committee may see fit to set.

SECTION 11. ASSOCIATES

Associates shall include students, nonprofessional persons interested in tropical medicine, and members of the families of professional members. Associates will have the privilege of attending general and sectional meetings, but will not have the right to participate in discussions or to vote. Associates shall have the privilege of purchasing a copy of the proceedings on payment of the specified additional fee at the time of registration.

Membership will be granted only on receipt of proper application, accompanied by the necessary subscription fixed by the Intersociety Committee for the appropriate class of membership. The Organizing Committee reserves the right to exclude from membership any person, persons, or organizations regarded as ineligible or undesirable.

OFFICERS OF THE CONGRESSES

SECTION 12. GENERAL

The officers of the Congresses shall be the following:

A Temporary President

The President

Three Vice Presidents

The Secretary General and Deputy Secretary General

A Chairman, two Vice Chairman, a Secretary and an Assistant Secretary for each section.

A temporary President shall be appointed by the President of the United States officially to open the Congresses; he shall serve until the President and Vice Presidents are elected by the Congresses at their opening session. The Secretary General and Deputy Secretary General shall be appointed by the President of the United States.

The section officers shall be chosen by the respective Sections with the exception of the Conveners who shall be selected by the Organizing Committee.

Honorary Presidents and Vice Presidents may be elected at the opening of the Congresses on the nomination of the Organizing Committee, and each section may choose Honorary Chairmen at a section meeting.

SECTION 13. THE PRESIDENT

The President of the Congresses shall preside over the general sessions of the Congresses and shall conduct the meetings in accordance with the usual par-

liamentary procedure and the rules of the Congresses. It shall be his duty to see that the rules of the Congresses are observed.

Should the President be unable to preside, the Vice Presidents shall take the chair in rotation.

SECTION 14. SECTION CHAIRMAN

Each Chairman of a Section shall preside at the meetings of that Section while the Congresses are in session. He may during the meetings temporarily vacate the chair in favor of one of the elected Honorary Chairmen or Vice Chairmen of the section.

The Chairman of a Section shall be responsible for the prompt transmission of the resolutions of the Section or its formulated wishes to the Secretary General of the Congresses.

Should the Chairman of a Section be unable to preside at the meetings, one of the Vice Chairmen shall take his place.

SECTION 15. SECTION SECRETARIES

It shall be the duty of the Secretary of a Section to maintain complete and detailed records of the proceedings of the Section, including a file of the reports or scientific papers submitted, a transcript of discussions, resolutions adopted, and any special business transacted by the Section. At the close of the Congresses, the Secretary of a Section shall deliver such records to the Secretary General of the Congresses for use in the preparation of the Report of the Proceedings.

SCIENTIFIC AND GENERAL MEETINGS

SECTION 16. LIST OF SECTIONS

There shall be formed the following sections covering the several important fields of tropical medicine and hygiene:

- I. Research and Teaching Institutes
- II. Tropical Climatology and Physiology
- III. Bacterial and Spirochetal Diseases
- IV. Virus and Rickettsial Diseases
- V. Malaria
- VI. Helminthic Diseases
- VII. Protozoan Diseases
- VIII. Nutritional Diseases of the Tropics
- IX. Tropical Dermatology and Mycology
- X. Tropical Veterinary Medicine
- XI. Public Health
- XII. Medical and Veterinary Entomology

SECTION 17. SUBJECTS AND PAPERS FOR SECTION MEETINGS

The Organizing Committee, through the Committee on Program, shall decide the subjects to be presented in the general sessions and in each section, and shall

invite for the presentation of each subject one or more persons conversant with that subject.

SECTION 18. JOINT MEETINGS AND SUB-SECTIONS

Each section shall have the privilege of dividing itself into sub-sections when such an arrangement seems desirable, or to form special committees to discuss especially important subjects. It shall be permissible also for two or more sections to unite in common meetings for the discussion of special subjects of interest to several sections.

PROGRAM, PAPERS, AND DISCUSSIONS

SECTION 19. GENERAL

The discussions of the Congresses shall take place in general meetings and in sectional meetings.

The opening and final sessions shall be general meetings. In addition, the Organizing Committee may arrange one or more general meetings (evening meetings) for discussion of subjects of general interest to members of the Congresses and may invite selected individuals to discuss such subjects.

The Organizing Committee shall outline the procedure for the opening and closing general meetings and shall select the program for such meetings. If other general meetings are held, the Committee on Program shall participate in the selection of the subjects for discussion.

No section meeting shall be held at the same time as a general meeting, but several sectional meetings may be held simultaneously.

The time and place of the several meetings shall be fixed and made known by the Organizing Committee or by the Secretary General.

SECTION 20. ATTENDANCE

All the above-mentioned meetings shall be open only to delegates, members, and associates of the Congresses and guests who may be specially invited by the Organizing Committee, except for such meetings as may be declared open to the public.

SECTION 21. LANGUAGES

The official languages of the Congresses shall be English, French, and Spanish.

SECTION 22. TRANSLATION OF PROPOSALS AND RESOLUTIONS

All formal proposals and resolutions will be submitted in one of the official languages and will be translated *in extenso* into the others. The discussions on such proposals and resolutions shall be translated only when desired by the majority of the members present, and then only in short summary.

SECTION 23. DATE FOR SUBMISSION OF PAPERS

It shall be obligatory upon every person invited to give a paper, to send an abstract, of not over 300 words, to the Organizing Committee by February 29,

1948, and send a copy of the full paper by March 31, 1948. Should such person fail to comply with this requirement, his paper may be removed from the program.

Anyone who has complied with the above provision, but who is unable to be present at the Congresses may select a substitute for the presentation of his paper. In the absence of the author and in the event that no substitute is selected, an abstract of the report may be read by the secretary of the Section.

SECTION 24. GENERAL RULES ON PAPERS

Papers shall be in one of the official languages of the Congresses. They shall be limited to 3,000 words and 20 minutes reading time except as otherwise agreed in special instances by the Program Committee.

SECTION 25. DISCUSSION OF PAPERS

Each person discussing any paper must confine his remarks to 3 minutes. Any member presenting such discussion shall submit to the Secretary General, if the discussion is in a general meeting, or to the section Secretary, if in a sectional meeting, a written abstract of his discussion not to exceed 500 words in one of the official languages.

SECTION 26. RESOLUTIONS

Resolutions dealing with general subjects shall be presented in writing to the Secretary General for reference to the Resolutions Committee prior to the time of the general meeting at which they are to be discussed. The presiding officer shall call for the presentation at the proper time.

Resolutions dealing with scientific parts of the program shall be presented in writing to the secretary of the proper section to be acted upon by that section and, in event of approval by that section, to be transmitted to the Secretary General for entering on the agenda of a general meeting.

The presentation of a resolution at any general or sectional meeting shall not exceed 5 minutes and the discussion of any such resolution by any member shall not exceed 3 minutes.

Action upon any resolution shall be decided by majority vote. In case of a tie vote, the presiding officer shall have the deciding vote.

CONCLUSION OF THE CONGRESSES

SECTION 27. RESOLUTIONS COMMITTEE AND INTERIM COMMITTEE

A Committee on Resolutions shall be appointed by the Temporary President. It shall review resolutions and proposals submitted in general and sectional meetings and decide whether and in what form these shall be submitted for consideration at the final meeting.

The general officers, the Chairmen of the various sections, and members of the Organizing Committee shall meet and prepare nominations for members of the Interim Committee of the Fifth International Congresses on Tropical Medicine and Malaria, for approval of the Fourth Congresses at the final meeting.

SECTION 28. REPORT OF THE CONGRESSES

On conclusion of the Congresses, the Organizing Committee shall cause a general report to be made of the organization and proceedings of the Congresses. The report shall be printed and distributed gratuitously to all Official and Institutional Delegates, and other professional Members of the Congresses, and such Associates as have paid the additional fee mentioned in Section 11 above.

SECTION 29. ARCHIVES OF THE CONGRESSES

Following liquidation of the business of the Fourth Congresses, copies of appropriate documents and records shall be turned over by the Secretary General of the Congresses to the Secretary of the Interim Committee, who shall maintain custody of them until his successor is elected.

FINANCIAL ADMINISTRATION

SECTION 30. REVENUES

The revenues of the Congresses, other than governmental funds, shall consist of (a) membership fees and (b) voluntary contributions from individuals, firms, corporations, or organizations. The funds under (b) shall not be subject to Sections 31 and 32 unless the donors have made the contrary a condition of their gifts.

SECTION 31. SURPLUS AND DEFICIT

The Intersociety Committee through its Committee on Finance shall be responsible for the careful administration of receipts and expenditures.

If the revenue of the Congresses does not suffice to cover the expenditures, the deficit will not be charged against succeeding Congresses.

Should any surplus remain after all debts and obligations of the Congresses have been satisfied, the Intersociety Committee shall take charge of this surplus and give notice to the Interim Committee. The latter shall take into consideration any proposal that may be made by the Intersociety Committee and shall report thereupon to the next Congresses, at the same time submitting a reasoned proposal as to the use of the surplus. The surplus may be used to pay the expenses of administration of the Interim Committee, but may not be employed to defray the expenses of subsequent Congresses.

SECTION 32. AUDIT

When the business of the Congresses has been completed, at the latest within 2 years, the Committee on Finance shall examine the accounts of the Intersociety Committee funds, which shall be subject to audit, and shall publish a report of the same in journals dealing with tropical medicine in those countries from which the largest number of members have been drawn.

SECTION 33. ABSTRACT OF ACCOUNTS

Abstract of the accounts shall be furnished the Department of State, the organizations represented in the Intersociety Committee, and the Interim Committee for placing in the archives.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgement must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

- da Costa Lima, A., e Hathaway, C. K. *Pulgas: Bibliografia, catalogo e animais por elas injetados.* (Pests: Bibliography, catalogue of species and hosts.) Monografias do Instituto Oswaldo Cruz, No. 4, 1946, Imprensa Nacional, Rio de Janeiro, Brasil.
- Sayers, R. R., and Davenport, Sara J.: *Copper and Health.* Issued by: Copper and Brass Research Ass'n, 429 Lexington Ave., New York 17, N. Y.
- Behrman, Howard T.: *Dermatologic Clues to Internal Disease.* 165 pp., 118 illus. Grune and Stratton, New York, 1947. \$5.00.
- Hull, Thomas G. (With numerous collaborators) *Diseases Transmitted from Animals to Man.* 571 pp., 75 illus. Charles C. Thomas, Springfield, Illinois, \$10.50.
- HARRI L. ALEXANDER. *Synopsis of Allergy*, 2nd ed., 235 pp., 22 illus. St. Louis, Mo., 1947. C. V. Mosby Co.
- F. J. PINKERTON, M.D., Chairman, Public Health Committee. *The Health Story in Hawaii*, pp. 111. Illus. Chamber of Commerce, Honolulu, 1947.
- FRANKLIN H. TAYLOR. *Communicable Diseases*, 2nd ed. 792 pp., 95 illus., 13 color plates. St. Louis, Mo., 1947, C. V. Mosby Co.
- R. G. COCHRANE. *A Practical Textbook of Leprosy*, with a foreword by George R. McRoberts, XII + 283 pp. Illus. London, New York, Toronto, 1947. Geoffrey Cumberlege: Oxford University Press. \$11.50.
- Memoranda on Medical Diseases in Tropical and Sub-Tropical Areas.* Illus., pp. 396. 8th ed. 1946. London: His Majesty's Stationery Office, 7/6.
- PATRICK A. BRXTON. *The Louse; An Account of the Lice Which Infest Man, Their Medical Importance and Control*, 2nd ed. Pp. VIII + 161. 47 Illus. Baltimore, Md., Williams & Wilkins, \$3.25.

BOOK REVIEWS

P. C. FLU. *The Bacteriophage. A Historical and Critical Survey of 25 Years Research.* Acta Leidensia, Scholae Medicinali Tropicae, Vol. 17, 1946.

The 165 page review is concerned with the problem of the nature of bacteriophage. Is it a living, autonomous agent that parasitizes the bacterial cell or is it produced in the cell by some autocatalytic process? Some of the more pertinent questions and subjects reviewed in detail are: 1) Is phage produced by microbes in their defense of the micro-organism or is it a substance formed by the action of ferments on microbes? 2) Does phage originate by bacterial mutation or variation or should it be regarded as a stage in the life-cycle of bacteria? 3) Does phage occur as a consequence of microbial antagonism or as the result of disintegration of bacteria? 4) Is phage formed by the action of chemicals in microbes? 5) The "Splitter" doctrine of Bail. 6) Bacteriophage and spore bearing microbes. 7) Hypothesis on the origin of virus and phage built up after the discovery of the virus protein by Stanley.

The discussion is divided into fourteen chapters and presents protocols of numerous experiments performed in the author's laboratory in attempts to repeat work reported by others in support of the autocatalysis hypothesis. This was not merely a matter of shooting down clay pigeons despite the fact that analysis is easier than synthesis. A reader may finish the review and retain the impression that he has been presented an exercise in logic in which the truth of the matter depends on the interpretations of the facts considered. It further emphasizes the recurrent pitfalls of any research work, errors in observation or in logic and the dangers of reasoning by analogy. The present work was written in 1942 and its completion was prevented by the war and by the author's death. There are 304 references none later than 1939. A biographical sketch of Professor Flu is included in the volume.

JOHN F. KESSEL.

HOWARD T. BEHRMAN, M.D. *Dermatologic Clues to Internal Disease.* Grune & Stratton, New York. \$5.00.

In this one hundred and sixty-five page book with its one hundred and eighteen illustrations Dr. Howard T. Behrman of New York University College of Medicine presents in picture and word summary form the salient features of outstanding dermatologic manifestations, together with the underlying internal disease which forms the background of each. Published by Grune & Stratton, this small volume is appropriately titled.

That pathologic changes of the skin are often manifestations of underlying systemic disease is by no means a new concept, but is nevertheless a fact which needs further emphasis. An approach to diseases of internal medicine through their manifestations in the skin has practical significance, of course, for such superficial changes not infrequently are the chief complaint of the patient and are his reason for consulting the physician.

By thumbing through this book, observing the clear, graphic plates and trying to identify the disease which each represents, the physician may well test his personal knowledge. Although the diseases are arranged alphabetically, a need is felt, at times, for an index.

It would appear that the plate illustrating amebiasis has little practical significance and might well have been omitted, while an occasional plate such as that illustrating hereditary hemorrhagic telangiectasia leaves something to be desired. One wonders if plates in colors might not have added materially to the value of the book. Many figures, such as those which illustrate avitaminosis, endocrine disorders, leprosy and tuberculosis, are particularly vivid and carry with them the intended punch.

Taken as a whole, the book is well worth while. Many physicians will undoubtedly feel the need for becoming acquainted with "Dermatologic Clues" and will want to make themselves better detectives who are able to follow each clue to its solution.

WEBSTER MERRITT.

THOMAS G. HULL. *Diseases Transmitted from Animals to Man*. 3rd edition. 75 Illus. Pp. XVIII + 571. Springfield, Ill., Charles C. Thomas. \$10.50.

Human illness acquired from lower animals, either domesticated or wild, constitutes a formidable hazard. With the aid of fourteen collaborators, Dr. Hull presents a review of thirty diseases, chiefly infectious, which constitute a menace to man. Although some of these are of waning importance in the United States, the differentiation and recognition of others previously unknown, serves to maintain these risks at a high level. Furthermore, domesticated animals may participate in the spread of some characteristically human infections. Although the diseases reviewed are considered from the standpoint of bacteriology, pathology, clinical symptoms in animals, and diagnosis, especial emphasis is given to epidemiology and prevention, along which lines lie the greatest usefulness of the book. Therapy receives scanty attention. The revision required for this edition brings the information presented well down to date. It is likely the work will be more useful to public health administrators than to any other professional group.

MARK F. BOYD.

FRANKLIN H. TOP AND COLLABORATORS. *Communicable Diseases*. 13 color plates and 95 Illus. Pp. 992. 2nd ed. St. Louis, Mo., C. V. Mosby Co.

This work is largely based upon experience gained in the Herman Kiefer Hospital, and presumably reflects the methods for the management of infections in that institution. In this second edition it is greatly expanded through the incorporation of fourteen new chapters. The descriptions are vividly enhanced by a large series of excellent and well chosen illustrations in black and white as well as in color. Seven chapters are devoted to general considerations, and each of the remaining fifty three are devoted to individual infections. This naturally does not permit of an exhaustive consideration of any single disease but this limitation is in most instances well offset by conciseness and balance of presentation. As a consequence of this condensation, however, the presentation of those insect transmitted diseases considered is not comparably adequate. The book should prove to be very useful to the clinician or epidemiologist.

MARK F. BOYD.

AUTHOR INDEX

A

- Albright, Edwin C., with Tabern, D. L. and Gordon, H. S., 553
 Alving, Alf S., with Craige, B., Jones, R., Whorton, C. M., Pullman, T. N., and Eichelberger, L., 309
 Anderson, Charles R., and Roca-Garcia, M., 161
 Anderson, Hamilton H., Johnstone, H. G., and Hansen, E. L., 153
 Angulo, Juan J., and Spies, T. D., 317
 Arb, J. E., 483
 Avila, Carlos, and Varela, Gerardo, 663

B

- Ball, Gordon H., 301
 Baker, Roger D., 749
 Bang, F. B., and Hairston, N. G., 31
 Bartter, F. C., with Cowie, D. B., Most, H., Ness, A. T., and Forbush, S., 403
 Bauman, Preston M., with Wright, W. H., McMullen, D. B., Bennett, H. J., and Ingalls, J. W., 417
 Beck, Otis H., and Leavitt, S. S., 347
 Bennett, Harry J., with Hunter, G. W., Ingalls, J. W., and Greene, E., 597
 Bennett, Harry J., with Wright, W. H., McMullen, D. B., Bauman, P. M., and Ingalls, J. W., 417
 Blank, Harvey, 383
 Blumenthal, H. T., with Dutra, F. N., Paschal, H., and Kuhn, L. R., 711
 Bowers, Ralph, with Burchenal, J. E., and Haedicke, T. A., 699
 Bozicevich, John, with Donovan, A., Mazzotti, L., Diaz, A. F., and Padilla, E., 51
 Brackett, Sterling and Kenney, M., 493
 Bransfield, J. W., and Dorrance, G. M., 77
 Buchheit, J. R., with Kuntz, R. E., and Stirewalt, M. A., 691
 Burchenal, J. H., with Bowers, R. F., and Haedicke, T. A., 699
 Burgess, Robert W., and Young, M. D., 39
 Burrows, Robert B., with Morehouse, W. G., and Freed, J. E., 327
 Butler, Fred A., and Sapero, J. J., 111
 Butts, Donald C. A., 607

C

- Calero, M. Carlos, and Ortiz, O. P., 377
 Casini, G., with Soper, F. L., Knipe, F. W., Richl, L. A., and Rubino, A., 177

- Chenoweth, B. M., Jr., with Franks, M. B., and Stoll, N. R., 617
 Christenson, L. D., and Oman, P. W., Suppl. 91
 Congresses, Fourth International for Tropical Medicine and Malaria, 521, 779
 Cova-Garcia, Pablo, 525
 Cowan, Frank A., with McGregor, T., and Randolph, N. M., 67
 Cowie, D. B., with Bartter, F. C., Most, H., Ness, A. T., and Forbush, S., 403
 Craige, Branch, Jr., with Jones, R., Whorton, C. M., Pullman, T. N., Alving, A. S., and Eichelberger, L., 309
 Cutkomp, Lawrence K., and Hinman, E. H., 419

D

- Daines, W. P., with van Gelder, D. W., and Fischer, G. L., 225
 de Caires, P. F., 733
 Diaz, A., Francisco, with Bozicevich, J., Donovan, A., Mazzotti, L., and Padilla, E., 51
 Donovan, Anthony, with Bozicevich, J., Mazzotti, L., Diaz, A., and Padilla, E., 51
 Dorrance, G. M., and Bransfield, J. W., 77
 Dove, Walter E., 339
 Downs, W. G., with Harper, P. A., and Lisansky, E. J., Suppl., 69
 Dutra, Frank N., with Blumenthal, H. T., Paschal, H., and Kuhn, L. R., 711
 Dwinelle, James H., with Sheldon, A. J., Rein, C. T., and Sternberg, T. H., 633

E

- Eichelberger, Lillian, with Craige, B., Jones, R., Whorton, C. M., Pullman, T. N., and Alving, A. S., 309
 Ellis, John M., with Young, M. D., and Stubbs, T. H., 585
 Elmendorf, John E., Jr., 135
 Eyles, Don E., with Hunter, G. W., and Warren, V. G., 203
 Eyles, Don E., and Most, H., 201

F

- Felsenfeld, Oscar, 285
 Fischer, G. L., with van Gelder, D. W., and Daines, W. P., 225
 Flu, P. C., 525

Forbush, Scott, with Bartter, F. C., Cowie, D. B., Most, H., and Ness, A. T., 403
 Foshay, Lee, 233
 Franks, M. B., with Chenoweth, B. M., and Stoll, N. R., 617
 Freed, Joe E., with Burrows, R. B., and Morehouse, W. G., 327
 Freund, Jules, with Thomson, K. J., Sommer, H. E., and Walter, A. W., 79

G

Gersh, Isodore, with Tullis, J. L., Jenney, E., McLimans, W. F., and Vinson, J. W., 245
 Gilchrist, Huntington, and Ruegsegger, J. M., 683
 Gingrich, Wendell, with Schoch, E. W., Schwab, M., and Shepherd, C. C., 147
 Goldman, Leon, 561
 Gordon, Edgar S., with Albright, E. C., and Tabern, D. L., 553
 Greene, Eugene, with Hunter, G. W., Bennett, H. J., and Ingalls, J. W., 597
 Greif, Roger L., 131
 Grocott, Robert G., and Kean, B. H., 745

H

Haedicke, Thomas A., with Burchenal, J. M., and Bowers, R. F., 699
 Hairston, K. G., and Bang, F. B., 31
 Hansen, Eder L., with Anderson, H. H., and Johnstone, H. G., 153
 Harper, Paul A., with Lisansky, E. T., and Sasse, B. E., Suppl., 1
 Harper, Paul A., with Downs, W. G., and Lisansky, E. T., Suppl., 69
 Harper, Paul A., and Levine, N. D., Suppl., 119
 Henderson, Lowell L., 643
 Hinman, E. Harold and Cutkomp, L. K., 449
 Hodgson, Corrin H., 69
 Hoobler, Sibley W., 477
 Hood, Marion, 505
 Hubbard, Clarence A., 525
 Hunter, George W., III, with Eyles, D. E., and Warren, V. G., 643
 Hunter, George W., III, with Bennett, H. J., Ingalls, J. W., and Greene, E., 597

I

Ingalls, James W., with Hunter, G. W., Bennett, H. J., and Greene, E., 597
 Ingalls, James W., with Wright, W. H., McMullen, D. B., Bennett, H. J., and Bauman, P. M., 417

J

Jenney, Elizabeth, with Tullis, J. L., Gersh, I., McLimans, W. F., and Vinson, J. W., 245
 Johnstone, Herbert G., with Anderson, H. H., and Hansen, E. L., 153
 Jones, Ralph, Jr., with Craig, B., Whorton, C. M., Pullman, T. N., Alving, A. S., and Eichelberger, L., 309

K

Kean, B. H., and Grocott, R. G., 745
 Kenney, Michael, and Brackett, S., 493
 Knipe, F. W., with Soper, F. L., Casini, G., Riehl, L. A., and Rubino, A., 177
 Kuhn, L. R., with Blumenthal, H. T., Dutra, F. N., and Paschal, H., 711
 Kuntz, Robert E., with Stirewalt, M. A., and Buchheit, J. R., 691

L

Leavitt, Samuel S., and Beck, O. H., 347
 Levine, Norman D., and Harper, P. A., Suppl., 119
 Liberia, International Institute Tropical Medicine, 517
 Linduska, J. P., and Morton, F. A., 771
 Lisansky, E. T., with Harper, P. A., and Sasse, B. E., Suppl., 1
 Lisansky, E. T., with Downs, W. G., and Harper, P. A., Suppl., 69.

M

Manwell, Reginald D., with Russell, P. F., and West, L. S., 201
 Markell, Edward K., with Mullinger, P. E., and Schneider, D. J., 63
 Masillamini, S. G., 107
 May, Ella Louise, 129, 323
 Mazzotti, Luis, with Bozicevich, J., Donovan, A., Diaz, A. F., and Padilla, E., 51
 McCain, B. E., and Rigdon, R. H., 673
 McGregor, Ted, with Cowan, F. A., and Randolph, N. M., 67
 McLimans, W. F., with Tullis, J. L., Gersh, I., Jenney, E., and Vinson, J. W., 245
 McMullen, Donald B., with Wright, W. H., Bennett, H. J., Bauman, P. M., and Ingalls, J. W., 417
 Meiklejohn, Gordon, and Simpson, T. W., 727
 Mooser, Herman, 525
 Morehouse, William G., with Burrows, R. B., and Freed, J. E., 327

- Morgan, Banner Bill, 399
 Morton, F. A., and Linduska, J. P., 771
 Most, Harry, with Bartter, F. C., Cowie, D. B., Ness, A. T., and Forbush, S., 403
 Most, Harry, and Eyles, D. F., 211
 Mullinger, Paul E., with Markell, E. K., and Schneider, D. J., 63

N

- National Institute of Health, Division of Tropical Diseases, 661
 Nelson, E. Clifford, 545
 Ness, A. T., with Bartter, F. C., Cowie, D. B., Most, H., and Forbush, S., 403

O

- Oman, P. W., and Christenson, L. D., Suppl., 91
 Ortiz, O. Pedro, and Calero, M. C., 377
 Ottolina, Carlos, 603

P

- Padilla, Enrique, with Bozicevich, J., Donovan, A., Mazzotti, L., and Diaz, A. F., 51
 Palmer, Eddy D., 45
 Paschal, Howard, with Blumenthal, H. T., Dutra, F. N., and Kuhn, L. R., 711
 Pullman, Theodore N., with Craige, B., Jones, R., Whorton, C. M., Alving, A. S., and Eichelberger, L., 309

R

- Randolph, Neal M., with Cowan, F. A., and McGregor, T., 67
 Rein, Charles T., with Sheldon, A. J., Dwinelle, J. H., and Sternberg, T. H., 633
 Reimann, Hobart A., 503
 Ricketts, William E., 657
 Riehl, Louis A., with Soper, F. L., Knipe, F. W., and Rubino, A., 177
 Rigdon, R. H., and McCain, B. E., 673
 Roca-Garcia, Manuel, and Anderson, C. R., 161
 Rodriguez-Molina, Rafael, and Shwachman, H., 117
 Rubino, A., with Soper, F. L., Knipe, F. W., and Riehl, L. A., 177
 Rueggsegger, James, and Gilchrist, H., 683
 Russell, P. F., with West, L. S., and Manwell, R. D., 201

S

- Sapero, James J., 271
 Sapero, James J., and Butler, F. A., 111

- Sasse, B. E., with Harper, P. A., and Lisansky, E. T., Suppl., 1
 Schneider, Donald J., with Markell, E. K., and Mullinger, P. E., 63
 Schoch, Eugenia Worley, with Gingrich, W., Schwab, M., and Shepherd, C. C., 147
 Schwab, Marjorie, with Gingrich, W., Schoch, E. W., and Shepherd, C. C., 147
 Sheldon, Albert J., with Dwinelle, J. A., Rein, C. T. and Sternberg, T. H., 633
 Shepherd, Catherine C., with Gingrich, W., Schoch, E. W., and Schwab, M., 147
 Shrapnel, Bliss C., 527
 Shwachman, Harry, and Rodriguez-Molina, R., 117
 Simmons, James Stevens, 1, 11
 Simpson, Thomas W., and Meiklejohn, G., 727
 Sommer, Harriet E., with Thomson, K. J., Freund, J., and Walter, A. W., 79
 Soper, Fred L., with Knipe, F. W., Casini, G., Riehl, A., and Rubino, A., 177
 Spies, Tom D., and Angulo, J. J., 317
 Sternberg, Thomas H., with Sheldon, A. J., Dwinelle, J. H., and Rein, C. T., 633
 Stirewalt, M. A., with Kuntz, R. E., and Buchheit, J. R., 691
 Stoll, N. R., with Franks, M. B., and Chenoweth, B. M., 617
 Stubbs, Trawick H., with Young, M. D., and Ellis, J. M., 535
 Sulkin, S. Edward, and Wisseman, C. L., 463

T

- Tabern, D. L., with Albright, E. C., and Gordon, E. S., 553
 Taylor, R. M., and Waddell, M. B., 471
 Thomson, K. Jefferson, with Freund, J., Sommer, H. E., and Watter, A. W., 79
 Tullis, John L., 21
 Tullis, John L., with Gersh, I., Jenney, E., McLimans, W. P., and Vinson, J. W., 245

V

- van Gelder, D. W., with Daines, V. P., and Fischer, G. L., 225
 Varela, Gerardo, and Avila, C., 663
 Vedder, Edward B., 723
 Vinson, J. W., with Tullis, J. L., Gersh, I., Jenney, E., and McLimans, W. F., 245

W

- Waddell, Mary B., and Taylor, R. M., 471
 Walter, Annabel W., with Thomson, K. J., Freund, J., and Sommer, H. E., 79

AUTHOR INDEX

- Warren, Virginia G., with Eyles, D. E., and
Hunter, G. W., 203
Weller, Thomas H., 41
Welt, Louis G., 221
West, Luther S., with Russell, P. F., and
Manwell, R. D., 201
Whorton, C. Merrill, with Craige, B., Jones,
R., Pullman, T. N., Alving, A. S., and
Eichelberger, L., 309
Wisselman, Charles L., Jr., and Sulkin, S. E.,
463
- Woke, Paul A., 357
Wright, Willard H., with McMullen, D. B.,
Bennett, H. J., Bauman, P. M., and
Ingalls, J. W., 417
- Y
- Young, Martin D., and Burgess, R. W., 39
Young, Martin D., with Ellis, J. M., and
Stubbs, T. H., 585
Young, Viola Mac, 293

SUBJECT INDEX

A

- Actinomycosis in a *Hydrochoerus isthmus* Goldman (Isthmian Capybara or Pongo), 377
- Aedes aegypti* Control in the Absence of a Piped Potable Water Supply, 733
- Amebiasis, Experimental Chemotherapy of, 153
- Oral Emetine in the Treatment of Intestinal, 527
- Anopheles Density Effected by the Pre-Season Spraying of Building Interiors with DDT in Kerosene, at Castel Volturno, Italy, in 1944-1945 and in the Tiber Delta in 1945, Reduction of, 177
- Anopheles maculipennis* Freeborni, The transmission of *Plasmodium malariae* by, 39
- Arthropods of Sanitary Importance in the Republic of Nicaragua, Central America, 357
- Atabrine (Quinacrine) Suppression of Malaria, Studies on, III. The Epidemiological Significance of Atabrine Suppression, 31
- Avian Malaria (*Plasmodium cathemerium*), Radical Cure of, with SN 8557, a Naphthoquinone Derivative, 147

B

- Benzene Hexachloride for Area Control of Trombiculid Mites, 771
- Biopsy by Transparency, The Rectoscopic. A New Diagnostic Method for *Schistosoma mansoni*, 603

C

- Carate, see Pinto, Mal de
- Carrión's Disease. A Study of the Incubation Period in Thirteen Cases, 657
- The Treatment of with Large Transfusions, 69
- Chiniofon using Radioactive Iodine, The Metabolism of, 553
- Cholera Epidemic in Chungking, Further Note on the Classification of Vibrios of the 1945, 503
- Chungking, Further Note on the Classification of Vibrios of the 1945 Cholera Epidemic in, 503

- Congresses on Tropical Medicine and Malaria, The Fourth International, 521, 779
- Costa Rica, Filarial Infection in, 607

D

- DDT for the Control of Malaria, Block Residual Spraying of Premises with, 449
- DDT Dust for the Control of Head Lice, 67
- DDT in Kerosene, Reduction of Anopheles Density Effected by the Pre-season Spraying of Building Interiors with, at Castel Volturno, Italy, in 1944-1945 and in the Tiber Delta in 1945., 177
- Dermanyssus gallinae*, Observations on the Laboratory Care, Life Cycle and Hosts of the Chicken Mite, 463
- Dimethylphalate Impregnated Clothing as Protection against Scrub Typhus, Use of, 221
- Dirofilaria immitis*, Reactions of Natives of Okinawa and of American Personnel, to Skin Tests with Test Antigen Prepared from Microfilariae of, 617
- Division of Tropical Diseases, National Institute of Health, 661

E

- Echinococcal Cysts of the Liver Followed by Deep X-Ray Therapy, The Evaluation of the Surgical Treatment of Recurrent, 77
- Emetine in the Treatment of Intestinal Amebiasis, Oral, 527
- Emetine Hydrochloride, Treatment of Trichuriasis with 'Enseals' of, 327
- Encephalitis, Japanese B, Sequelae of, 727
- Endamoeba histolytica*, Alcoholic Extract Medium for the Diagnosis and Cultivation of, 545
- Cysts, Agglutination of, 131
- The Significance of, in Stools of Individuals with Acute Diarrhea of Moderate Severity, 711
- Exoerythrocytic Parasites and the Tissue Reaction Caused by Blood-induced *Plasmodium gallinaceum* Infection in Chicks, The Distribution of, 21

F

- Filariasis Acquired in the South Pacific, The Periodicity of Microfilariae in two Patients with, 203

- Filarial Infection in Costa Rica, 607
 Florida, The Present Status of Hookworm Infection in, 505
 Folic Acid, A Case of Sprue Maintained on, 723
 Content of Foods Usually Consumed by Patients with Tropical Sprue, the Determination of, 317
 Fuadlin Therapy in 150 Cases of Schistosomiasis mansoni with a Follow up Study of 70 Cases, 117
- H
- Haemagogus equinus* as a vector of the virus, Studies on the Cyclic Passage of Yellow Fever Virus in South American Mammals and Mosquitoes. III. Further Observations on, 471
 Hookworm Infection in Florida, The Present Status of, 505
- I
- Iso-spora Hominis Infection in Man, 323
- J
- Japan, The Epidemiology of Schistosomiasis japonica in the Philippine Islands and. III. Surveys of Endemic Areas of Schistosomiasis japonica in Japan, 417
- L
- Leishmaniasis—Dermatological Aspects, Types of American Cutaneous, 561
 Leishmaniasis, Visceral, Complicated by Severe Anemia—Improvement Following Splenectomy, 699
 Liberia, Scene of New International Institute for Tropical Medicine, 517
 Lice, DDT Dust for the Control of Head, 67
 Lymph Node in Tropical Diseases, The, 483
- M
- Malaria, Block Residual Spraying of Premises with DDT for the Control of, 449
 Control, Preliminary Report on Field Experiments to Demonstrate Effectiveness of Various Methods of, 135
 Immunization of Ducks Against, by Means of Killed Parasites with or without Adjuvants, 79
 New Concepts in the Treatment of Relapsing, 271
 and Other Insect-Borne Diseases in the South Pacific Campaign, 1942-1945:
- I. General Aspects and Control Measures, Suppl., 1
 - II. Epidemiology of Insect-Borne Diseases in Army Troops, Suppl., 69
 - III. Entomology, Suppl., 91
 - IV. Parasitological Observations on Malaria in Natives and Troops, and on Filariasis in Natives, Suppl., 119
- Parasites, Premature Rupture in Schizogony; an Explanation for Multiple Infection of Red Blood Corpuscles with, 107
 Studies on Atabrine (Quinacrine) Suppression of. III. The Epidemiological Significance of Atabrine Suppression, 31
 Mapharsen (Chlorhydrate of 3-Amino-4 Oxarsenbenzen), in the treatment of Mal de Pinto or Carate, 663
 Metachloridine in Suppression Natural Infections with *Plasmodium malariae* and *P. falciparum* in British Guiana, The Effectiveness of, 493
 Microfilariae, The Cuticular Morphology of some Common, 233
 Mite, *Dermanyssus gallinae*, Observations on the Laboratory Care, Life Cycle, and Hosts of the Chicken, 463
- N
- Naphthoquinone Derivative, SN 8557, Radical Cure of Avian Malaria (*Plasmodium cathemerium*) with, 147
 Nicaragua, Central America, Arthropods of Sanitary Importance in the Republic of, 357
- O
- Ointments and Fabrics, Methods of Testing, to Determine their Effectiveness as Barriers to Schistosome Cercariae, 691
 Okinawa and of American Personnel, to skin tests with test antigen prepared from Microfilariae of *Dirofilaria immitis*, Reactions of Natives of, 617
Onchocerca volvulus, Intradermal and Complement Fixation Reactions Elicited by Various Antigens in Persons Infected with, 51
- P
- Pacific, The Periodicity of Microfilariae in Two Patients with Filariasis Acquired in the South, 203

Pacific Campaign, 1942-1945, Malaria and Other Insect-Borne Diseases in the South:

I. General Aspects and Control Measures, Suppl., 1

II. Epidemiology of Insect-Borne Diseases in Army Troops, Suppl., 69

III. Entomology, Suppl., 91

IV. Parasitological Observations on Malaria in Natives and Troops, and on Filariasis in Natives, Suppl., 119

Pacific vivax malaria in the American Negro, 111

Pamaquin (Plasmochin) in Mosquito-Induced vivax Malaria, Chesson strain, Clinical Standardization of, 309

Panama, Salmonella Infections in. Review of 219 Consecutive Hospital Cases Occurring in the 5 Year Period, 1942-1946, 643

Parasitic Infections in Naval Personnel, Intestinal, 63

Parasitologic Study of 400 Soldiers Interned by the Japanese, 129

Pasteurella tularensis in Fish, Experimental Studies with, 399

Pinto, Mal de, or Carate and its Treatment with Chlorhydrate of 3-Amino-4 Oxiarsenbenzen (Mapharsen), 663

Philippine Islands and Japan, The Epidemiology of Schistosomiasis japonica in the. III. Surveys of Endemic Areas of Schistosomiasis Japonica in Japan, 417

Piperonyl Butoxide, a New and Safe Insecticide for the Household and Field, 339

Plague: A Survey of Recent Developments in the Prevention and Treatment of, 683

Plasmodium cathemerium, Radical Cure of, Avian Malaria with SN 8557, a Naphthoquinone Derivative, 147

falciparum in British Guiana, The Effectiveness of Metachloridine in Suppressing Natural Infections with *Plasmodium malariac* and, 493

gallinacum Infection in Chicks, The Distribution of Exoerythrocytic Parasites and the Tissue Reaction Caused by Blood-Induced, 21

lophurae, Some Factors that Influence the Degree of Parasitemia in Ducks Infected with, 673

malariae, The Transmission of, by *Anopheles maculipennis* Freeborni. 39

malariac and *P. falciparum* in British Guiana, The Effectiveness of Metachloridine in Suppressing Natural Infections with, 493

relictum, Attempts to Cultivate the Mosquito Phase of, 301

Q

Quinacrine in Normal Subjects, Psychotic Reactions to the Ingestion of Large Doses of, 477

Studies on Atabrine Suppression of Malaria. III. The Epidemiological Significance of Atabrine Suppression, 31

S

Salmonella Infections in Panama. Review of 219 Consecutive Hospital Cases Occurring in the 5 Year Period, 1942-1946, 643

Salmonellosis in Different Climates, Polyvalent Antigens for the Diagnosis of, 285

Schistosome Cercariae, Method for Testing Ointments and Fabrics to Determine their Effectiveness as Barriers to, 691

Schistosomiasis japonica, 347

The Molluscan Intermediate Host and, III. Experimental Infection of *Oncomelania quadrasi*, The Molluscan Intermediate Host of *Schistosoma japonicum*, 597

in the Philippine Islands and Japan, The Epidemiology of. III. Surveys of Endemic Areas of Schistosomiasis in Japan, 417

Results of Certain Studies on the Stomach in, 45

mansoni, Fuadin Therapy in 150 Cases of with a Follow up Study of 70 Cases, 117

Infections, The Diagnosis of, Note on the Use of a Rectal Scraper, 41

The Rectoscopic Biopsy by Transparency. A New Diagnostic Method for, 603

Shigella Carriers with Special Reference to Their Therapy, Including the Use of Streptomycin, 225

The Geographical Distribution of, 293

SN 8557, a Naphthoquinone Derivative, Radical Cure of Avian Malaria (*Plasmodium cathemerium*) with, 147

Sporotrichosis, Experimental, in Mice, 749

Sprue, A Case of, Maintained on Folic Acid, 723

T

- Tartar Emetic Administered to Human Subjects, The Fate of Radioactive. 1. Blood Concentration and Excretion Following Single and Multiple Intravenous Injections, 403
- Toxoplasmosis, Asymptomatic, 745
- Trichuriasis with 'Enseals' of Emetine Hydrochloride, Treatment of, 327
- Trombiculid Mites, Benzene Hexachloride for Area Control of, 771
- Tropical Medicine and the Challenge of Global Peace, 11
- Tropical Medicine and the Challenge of Global War, 1
- Tropical Phagedenic Ulcer (Vincent's Ulcer), 383
- Tsutsugamushi Disease (Scrub Typhus) in Swiss Mice and Macacus Rhesus Monkeys and the Report of One Human Case Acquired in the Laboratory, Tissue Pathology of Experimental, 245
- Typhus (Scrub) in Swiss Mice and Macacus Rhesus Monkeys and the Report of one Human Case Acquired in the Laboratory, Tissue Pathology of Experimental Tsutsugamushi Disease, 245

V

- Vincent's Ulcer, Tropical Phagedenic Ulcer, 383
- Vivax Malaria in the American Negro, Pacific, 111
- Vivax Malaria, Chesson Strain, Clinical Standardization of Pamaquin (Plasmochin) in Mosquito-Induced, 309
- Vivax Malaria induced in Neurosyphilitic Patients, Some Characteristics of Foreign, 585

W

- Wuchereria bancrofti* to Mosquitoes of the United States, Infectivity of Pacific Island, 211

Y

- Yaws, Evaluation of Penicillin in the Treatment of. Final Report, 633
- Yellow Fever Virus, The Reaction of Woolly Opossums (*Caluromys laniger*) to, 161 in South American Mammals and mosquitoes, Studies on the Cyclic Passage of. III. Further Observations on *Hae-magogus equinus* as a Vector of the Virus, 471

The anatomic evidence of injury is not marked in any stage of the disease. At the site of natural or artificial intradermal inoculation a superficial non-specific skin ulceration occurs. (Subcutaneous inoculation will not produce ulceration.) Regional lymphadenitis accompanies the ulcer. Both subside and the ulcer heals by scar formation. Perivascular lymphoid-macrophage collections, a few small scattered foci of necrosis, and adherence of white blood cells to the endothelial lining of capillaries and smaller blood vessels sometimes associated with slight perivascular edema may be the only signs of injury. The scanty anatomic changes are in marked contrast to the changes seen in other rickettsial diseases such as Rocky Mountain spotted fever and Old World typhus fever. In tsutsugamushi disease it appears from the endothelial stickiness and perivascular edema that the endothelium is damaged and that perivascular collections of lymphocytes are present as defense mechanisms in response to occult injury. In Rocky Mountain spotted fever and in European typhus, destruction of vascular endothelium, thrombus formation, infarction and necrosis are evident. These lesions were not observed in experimental tsutsugamushi disease in mice, monkeys or in the human case.

SUMMARY AND CONCLUSIONS

It has been demonstrated that experimental tsutsugamushi disease in Swiss mice and *M. rhesus* monkeys in the terminal stages is pathologically similar to the terminal stages of the disease in man. It is a disease in which there is widespread involvement of tissues of mesenchymal origin, an involvement that depends on lymphoid-macrophage proliferation, mobilization, and invasion. The lymphoid elements show signs of hyperplasia early in the disease. In Swiss mice, the spleen is the first organ to react, increased mitotic activity being noted on the second or third day post inoculation followed by hyperplasia. One or two days later the generalized systemic involvement begins, manifested by the appearance of the lymphoid-macrophage cells in the stroma of the various organs. Endothelial stickiness is prominent early in mice and monkeys but gradually subsides in the monkey as the convalescent period is reached. In neither experimental animals or man is the endothelium destroyed, but sometimes it appears swollen and hypertrophied. Likewise the blood vessel walls may be infiltrated with lymphoid-macrophage cells, but only rarely is the reaction severe enough to cause tissue necrosis. Perivascular cell collections increase in number and size as the disease progresses and persist during the healing period. Small focal necroses in the parenchyma of the various organs may be seen in the advanced stage of the disease particularly in the vicinity of the large cell collections. However, because of the lack of truly pathognomonic lesions, diagnosis of the disease should depend not only on tissue changes, but should include observations on the history of exposure to the mite vector, demonstration of the presence of the organism by blood or ground spleen passage to animals and by staining of organ impression smears, and finally, by observation of the trend of Proteus OX-K agglutination titers.

ACKNOWLEDGMENTS

The support and cooperation of the following are gratefully acknowledged: Commander C. F. Geschickter, MC(S), USNR, for supplying the tissue blocks on the human case presented, and for many valuable suggestions; Lieutenant C. W. Grant, H(S), USNR, for repeated counsel, and for many valuable suggestions; Lieutenant (jg) M. F. Morales, H(S), USNR, for the analysis of the data incorporated in figure 1; animal work: Micetich, A., PhM2c, V6, USNR, Clark, D. L., PhM2c, V6, USNR; technical work: Lewart, R. M., HA1c, USNR.

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